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Reappearance of Spawning Atlantic Herring (*Clupea harengus harengus*) on Georges Bank: Population Resurgence not Recolonization

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Spawning Atlantic herring (*Clupea harengus harengus* L.) have been recorded on Georges Bank for the first time since the collapse in 1977 of what had once been the largest herring fishery in the Northwest Atlantic. The reappearance after almost a decade could have resulted from resurgence of a residual extant Georges Bank population or from recolonization by fish from neighboring spawning groups. Three independent lines of evidence are consistent with the resurgence hypothesis: the Georges bank fish differed in age composition and isozyme characteristics from neighboring populations, and the reappearance was later than expected for recolonization. The degree of isozyme variability and of mitochondrial DNA lineage diversity gave no indication that stochastic events influenced the genetic structure of the herring population at Georges Bank after the collapse. The persistence of this population, in spite of considerable potential for recolonization, supports the discrete population concept in herring.

Des harengs de l'Atlantique (*Clupea harengus harengus* L.) en phase de frai ont été observés dans le banc Georges pour la première fois depuis l'effondrement, en 1977, de ce qui avait autrefois été la plus importante pêcherie de harengs du nord-ouest de l'Atlantique. La réapparition de ce poisson, après près d'une décennie, pourrait être attribuable à la réapparition d'un prolongement résiduel de la population du banc Georges ou à la recolonisation par des poissons des groupes reproducteurs avoisinants. Trois éléments de preuve indépendants viennent étayer la première hypothèse : les poissons du banc Georges affichaient en effet une composition d'âge et des caractéristiques d'isozymes différentes des populations avoisinantes et la réapparition des poissons s'est faite plus tard que ce à quoi on pouvait s'attendre pour la recolonisation. Le degré de variabilité des isozymes et la diversité de lignée de l'ADN mitochondrial ne permettent pas de supposer que des événements stochastiques auraient influencé la structure génétique de la population de harengs du banc Georges après son effondrement. La persistance de cette population, malgré le potentiel de recolonisation considérable, vient étayer l'hypothèse d'une population discrète chez les harengs.

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A fishery for Atlantic herring (*Clupea harengus harengus*) on Georges Bank began in 1961 and yielded 2.7 million t before crashing in 1977 after a classic "boom and bust" pattern (Fig. 1). For several years, there was virtually no sign of either adults or larvae from a spawning population that had once been estimated at 1.14 million t in size (Anthony and Waring 1980; Hennemuth and Rockwell 1987). The commercial loss was felt not only in the collapse of the large adult fishery, but also in the decrease of the coastal weir fishery in Maine and New Brunswick, which is assumed to have been partially dependent on juveniles from Georges Bank (Sinclair et al. 1985). The socio–economic implications of the collapse prompted international meetings to examine the possibility of re-establishing herring on Georges Bank (Graham 1988).

Recent surveys have indicated reappearance of herring on Georges Bank, including documentation of spawning in the last 2 yr (1986–87). The reappearance, almost a decade after collapse of the fishery, raises the question of whether it has been

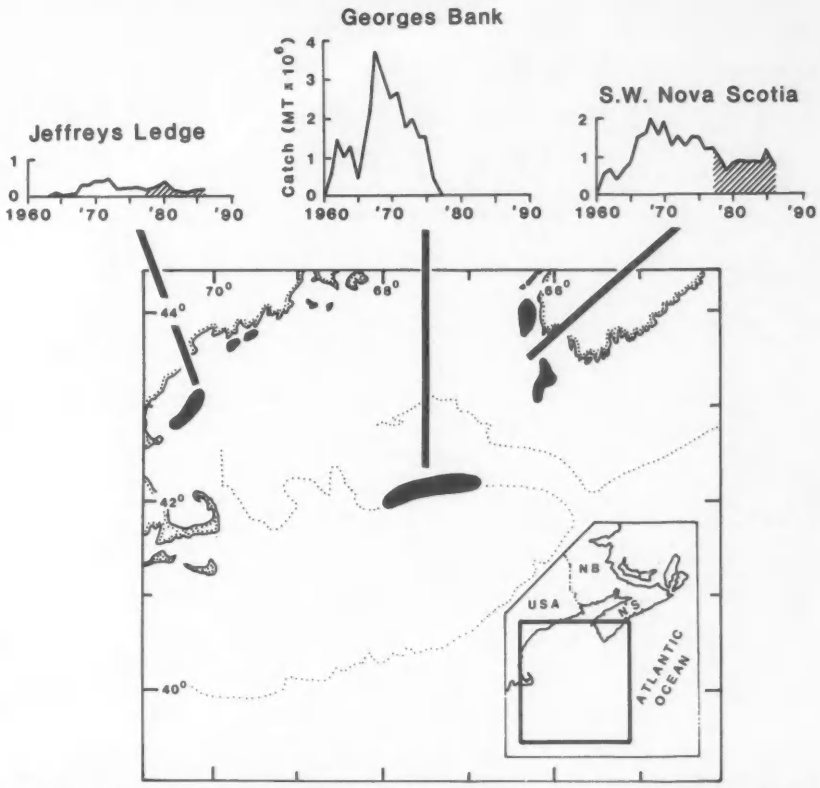

FIG. 1. Major spawning locations (shaded) for Atlantic herring (*Clupea harengus harengus*) of Georges Bank and neighboring populations in the Gulf of Maine and Bay of Fundy. Dotted line indicates the position of the 100-m contour. Insets show historical landings for the commercial fisheries of the three management units; shaded portion indicates the activity in neighboring fisheries after the collapse of the Georges Bank stock. The three management units in the Gulf of Maine recognize major spawning grounds but subsume smaller spawning locations.

the result of resurgence of some remnant of the original Georges Bank spawning population, or recolonization of the Bank by a neighboring population. The answer to this question is critical to interpretation of herring stock structure generally. Regularity of spawning (both geographic and temporal) (Sinclair and Tremblay 1984), tag evidence for homing (Harden Jones 1968; Wheeler and Winters 1984), differential population dynamics of neighboring groups (Sinclair and Iles 1985), and discrete larval distributions (Iles and Sinclair 1982) all suggest that herring spawning units are distinct populations, i.e. self-sustaining and geographically and genetically discrete. On the other hand, the lack of demonstrable differences in genetic (particularly isozyme) characteristics and weak results from traditional stock identification methods have been interpreted as indicating significant gene flow among neighboring spawning aggregations, and it has been suggested that herring populations are artificial subdivisions of a larger gene pool and are of no evolutionary or taxonomic significance (Smith and Jamieson 1986).

In this paper we chronicle the reappearance of herring on Georges Bank and discuss biological attributes of these fish and of the reappearance which are of relevance to this resurgence/recolonization question.

Materials and Methods

Evidence for the reappearance of herring on Georges Bank has been compiled primarily from directed surveys for larvae (oblique bongo tows) and from the appearance of adult herring in both Canadian and United States research bottom trawl surveys. Additional observations have come from juvenile gadid surveys (IYGPT trawl) and exploratory trips by the commercial fishery (Table 1).

Samples of spawning fish taken from the northeast peak and northern edge of Georges Bank in the fall of 1986 on Canadian and United States bottom trawl surveys (Table 1) provided the first chance to compare the stock characteristics associated with the reappearance. One hundred and forty-four fish (33 from RV *Albatross* cruise 86–05(II); 111 from RV *Alfred Needler* cruise 71) were aged by counting otolith annuli under low magnification and their phenotypes characterized for the isozymes *phosphoglucose isomerase-2* (*PGI-2*; EC 5.3.1.8), *lactate dehydrogenase-2* (*LDH-2*; EC 1.1.1.27) and *phosphoglucomutase-2* (*PGM-2*; EC 2.7.5.1) using horizontal starch gel-electrophoresis as in Kornfield et al. (1982). Eggs from 26 female herring (13 from each cruise) were characterized for mitochondrial DNA (mtDNA) cleavage phenotypes. mtDNAs

TABLE 1. Chronology of the reappearance of herring on Georges Bank.

Date	Observation	Vessel/Location
12 June 1984	Small juveniles (n>200) taken by midwater (IYGPT) trawl on Canadian research survey. All from 1983 year-class; mean length = 60.5 mm.	R/V *Alfred Needler* cruise 29. One set at 42°02′N, 66°59′W.
24 Feb. 1986	Herring (n = 213; non-reproductive) taken by ground trawl on U.S. groundfish survey. All from 1983 year-class.	R/V *Delaware* cruise 86-02. At four sets around 41°49′N, 66°50′W.
5–12 Mar. 1986	Herring (n = 786; non-reproductive) taken by ground trawl on Canadian groundfish survey. Length range 15–26 cm (mean = 19.5 cm) indicates 1983 year-class.	R/V *Alfred Needler* cruise 59. 19 sets.
19–20 May 1986	First successful commercial purse seiner catch.	M/V *Lady Melissa*. Sets of approx. 2 t (42°04′N, 66°30′W) and 68 t (42°05′N, 66°35′W) by purse seine.
14–24 Oct. 1986	Adult herring (n = 43; reproductively active) taken by ground trawl on U.S. groundfish survey. All from 1983 year-class.	R/V *Albatross* cruise 86-05 (II). Along northern edge of Georges Bank
29 Oct.–5 Nov. 1986	Adult herring (n = 111; reproductively active) taken by ground trawl on Canadian groundfish survey. Almost entirely 1983 year-class.	R/V *Alfred Needler* cruise 71. 13 sets along 50-fathom contour of northeast peak.
3–11 Nov. 1987	Adult herring (n = 396; reproductively active) taken by ground trawl, and larvae (in 19 sets; in significant numbers (n>200) in four sets) taken by bongo net in Canadian research survey.	R/V *Lady Hammond* cruise 181.

were extracted from individual frozen samples using phenol/chloroform, precipitated with ethanol, rehydrated, and subjected to digestion with a battery of six restriction enzymes (*Apa*I, *Bgl*II, *Bst*EII, *Eco*RI, *Eco*RV, *Xho*I). These were known to generate fragments of variable lengths from herring mtDNAs in previous studies. Cleavage fragment products were visualized by ethidium bromide staining of 1% agarose gels after horizontal electrophoresis as in Kornfield and Bogdanowicz (1987).

Results and Discussion

The absence of herring after collapse of the Georges Bank fishery was confirmed in a number of research surveys by both Canada and the United States. Surveys for larvae and for adults during the traditional autumn spawning time recorded almost no herring between 1977 and 1984 (Smith and Johnson 1986; Azarowitz and Grosslein 1987). The reappearance was first noted as postlarval herring taken on a research survey in the spring of 1984 (Table 1). Research surveys began detecting prespawning adult herring in the spring of 1986 and the first spawning adults in the fall of 1986; these fish were almost exclusively 3-yr-olds (1983 year-class). Greater numbers of spawning adults were documented again in the fall of 1987, as were substantial numbers of larvae.

Herring spawn demersally at discrete sites to which they are assumed to home (Wheeler and Winters 1984; Sinclair et al. 1985). Within the Gulf of Maine, major spawning sites are located on the northern edge of Georges Bank, in coastal waters of New England (Jeffreys Ledge) and off southwest Nova Scotia (Fig. 1). Spawning times are regular within each location, but vary among locations (Sinclair and Tremblay 1984). Larvae are pelagic but remain within population-specific larval retention areas, often in the general area of spawning, for several months (Iles and Sinclair 1982; Sinclair and Tremblay 1984). In the Gulf of Maine and Bay of Fundy, juveniles (< age 3) of all spawning groups are believed to aggregate and mix in coastal embayments (Anthony and Fogarty 1985; Sinclair et al. 1985). A portion of each year-class matures at age 3, most others at age 4, at which time individuals join adult migratory patterns between spawning, overwintering, and summer feeding areas. Except at spawning and during the first few months of the larval phase, there may be considerable mixing among populations at all phases of the life history (Sinclair and Iles 1985; Sinclair 1988).

The historical Georges Bank herring stock had the characteristics of a discrete population. It had regular spawning at predictable locations, a discrete larval distribution and, as evident in the dramatic fishery collapse, different population dynamics from neighboring groups. Although the population crashed before major tagging studies, fishery information indicated a regular migration pattern from the spawning grounds on Georges Bank to overwintering areas south of Cape Cod.

Three independent lines of evidence suggest that the reappearance of herring on Georges Bank was due to resurgence of an extant Georges Bank population rather than to recolonization from elsewhere. First, the reappearance was by a single year-class (Table 1). As 3-yr-olds, the 1983 year-class presumably spawned on Georges Bank for the first time in 1986. The age composition on Georges Bank differed significantly (p<0.01) from that in neighboring populations which contained larger numbers of older year-classes (Fig. 2). Under recolonization, the age composition of Georges Bank herring should have been similar to that of surrounding regions. Second, isoenzyme analysis of tissues from adult Georges Bank herring revealed differences from other stocks in the Gulf of Maine. Phenotypes of 144 reproductive herring were characterized for *phosphoglucose isomerase-2* (*PGI-2*; EC 5.3.1.8), the most polymorphic isoenzyme system in Atlantic herring (Andersson et al. 1981; Kornfield et al. 1982; Grant 1984). Electromorph frequencies of PGI^{-75} (0.007 ± 0.005 SE) and PGI^{40} (0.073 ± 0.015) in this sample differed significantly (P<0.01) from all previous Gulf of Maine samples (0.129 ± 0.043) and (0.002 ± 0.001), respectively (Kornfield et al. 1982; Grant 1984). Further, two individuals from this sample were heterozygous for PGI^{35}, a unique electromorph not observed elsewhere in the Gulf of Maine (n = 592), or indeed in any fish previously analyzed from the Northwest Atlantic (n = 1249) (Kornfield et al. 1982; Grant 1984). Under recolonization, homogeneity of electromorph frequencies is expected among spawning units. Third, the reappearance of Georges Bank herring did not occur for several years during a time when neigh-

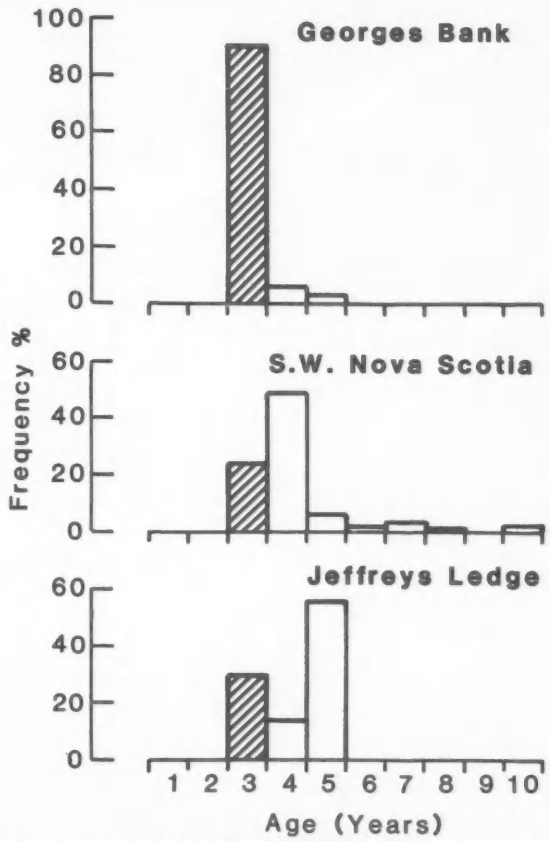

FIG. 2. Age composition of Atlantic herring (*Clupea harengus h. harengus*) samples from Georges Bank and adjacent spawning grounds in the Gulf of Maine (Jeffreys Ledge and southwest Nova Scotia) in 1986. Age was determined by counting otolith annuli under low magnification. Shading identifies the 1983 year-class. Samples for southwest Nova Scotia and Jeffreys Ledge were from commercial purse seine fisheries on spawning aggregations. Georges Bank samples were from research bottom trawls. Catch data: Georges Bank — Oct./Nov. 1986 ($n = 140$); southwest Nova Scotia — Aug./Sept. 1986 ($n = 191$); Jeffreys Ledge — Sept. 1986 ($n = 30$).

boring populations supported substantial fisheries (Fig. 1). Under recolonization, reappearance should have happened earlier. In addition, the group which has reappeared exhibits characteristics (location and timing of spawning) which match those of the historical Georges Bank population and differ from those of neighboring populations. Herring in spawning condition were taken on Georges Bank in late October and early November 1986 (Table 1). Historical spawning on Georges Bank peaked in the period Oct. 5–23. Spawning time on Jeffreys Ledge (peak Sept. 29–Oct. 25) overlaps that of Georges, but spawning in southwest Nova Scotia is earlier (Aug. 25–Sept. 10) (Sinclair and Tremblay 1984).

All of this evidence indicates resurgence. Although the possibility of limited mixing among populations cannot be discounted, the genetic characteristics of the Georges Bank herring are unique among Gulf of Maine populations.

It is not possible to estimate what the population size of Georges Bank herring was after collapse or prior to reappearance. However, two independent observations suggest that abundance did not decrease to a level where genetic drift or founder effects influenced population characteristics. First, the level of isozyme variability in the Georges Bank sample is not depressed; levels of heterozygosity (H) and effective number of alleles (N_e) (Nei et al. 1975) for *PGI-2* ($H = 0.499, N_e = 1.772$), *lactate dehydrogenase-2* ($H = 0.092$, $N_e = 1.102$) and *phosphoglucomutase-2* ($H = 0.480$, $N_e = 1.923$) are statistically equivalent to those observed in other populations of Northwest Atlantic herring (Kornfield et al. 1982; Grant 1984). Second, the level of mitochondrial DNA lineage diversity (h) (Gyllensten and Wilson 1987) in Georges Bank herring ($h = 0.846$) is equivalent to that observed for other spawning groups in the Northwest Atlantic ($h = 0.899 \pm 0.011$) (Kornfield and Bogdanowicz 1987). mtDNAs from 26 female herring collected on Georges Bank were characterized for cleavage phenotypes with six hexanucleotide restriction endonucleases. Of 13 composite phenotypes observed, 11 had been noted in other population samples in the Gulf of Maine (I. Kornfield, unpubl. data). Because of the small number of individuals examined at some of these localities, we do not attach significance to the two unique composites found at Georges Bank. These data suggest that stochastic events have not recently influenced population structure at Georges Bank, and that during the collapse the population remained genetically robust, though commercially inviable.

The concept of stock "collapse" has economic meaning, but is ambiguous biologically; collapse need not be equivalent to extinction. From the perspective of the commercial fishery, the Georges Bank population collapsed in 1977 because herring were not available in commercial quantities. Increased fishing effort or gear modifications might have resulted in herring capture, but these changes were not economically justified. Similarly, research efforts after the collapse were lower and a persistent small group could have been overlooked.

The demise of the Georges Bank population is thought to have been the result of overfishing, combined with a series of poor recruitment years (Anthony and Waring 1980; Grosslein 1987). Other populations in the Gulf of Maine show coincidence of strong and weak year-classes (Anthony and Waring 1980; Anthony and Fogarty 1985). The period directly after collapse, when the Georges Bank population showed no evidence of recovery, coincided with several years of low or average recruitment in neighboring populations (Anthony and Fogarty 1985; Stephenson et al. 1987). During that time, variables which influenced recruitment were negatively impacting all herring in the Gulf of Maine; perhaps only recently has there been generally good recruitment (as documented in neighboring southwest Nova Scotia (Stephenson et al. 1987)) providing a chance for the Georges Bank population to recover.

It is difficult to predict the rate of recovery of the Georges Bank population. Surveys in 1987 indicate an increased spawning population size over 1986, and the presence of substantial numbers of larvae. Estimates just prior to the collapse indicated a population biomass of 2–300 000 t (Anthony and Waring 1980) (although some considered this to be an overestimate) which was insufficient to maintain the viability of the population under fishing pressure and a period of low recruitment. Clearly, opening of the fishery should depend upon a minimum population which takes into account previous (pre-collapse) estimates as well as consideration of biological characteristics such as age distribution in the context of the management strategy being adopted.

Reappearance of the Georges Bank population provides a rare chance to test prevailing views on several aspects of herring biology and stock structure, including changes in growth and other biological parameters with increasing stock size. To further support our inferences regarding autonomy of the Georges Bank population, we suggest continued monitoring of both genetic and life history characteristics (such as year-class configuration). Comparison of these attributes with neighboring populations will permit assessment of our thesis. This research should also focus on the stock structure of other discrete spawning locations, particularly Nantucket Shoals.

The Gulf of Maine is small relative to the migratory range of juvenile and adult herring, and to the potential for dispersal during a pelagic larval phase of several months. That the reappearance seems to have been due to resurgence rather than recolonization is evidence for population persistence in spite of a complex life history which includes a long larval phase. Support for the discrete population concept in herring is significant to development of robust life history theory (Iles and Sinclair 1982; Sinclair et al. 1985; Smith and Jamieson 1986; Sinclair 1988).

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Effect of Climatic Warming on the Southern Margins of the Native Range of Brook Trout, *Salvelinus fontinalis*

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Stream inventories of brook trout (*Salvelinus fontinalis*) habitat show that the minimum altitude of a brook trout stream in the southern part of the native range rises steadily from sea level at about 39°12′N, to approximately 640 m at about 34°40′N at the southern margin of the range. Using this empirical lower stream boundary and a statistical model of the influence of altitude and latitude on groundwater temperature, I suggest that the lower altitudinal margin of the southern part of the native range is shaped by the 15°C groundwater isotherm. I used the climate warming scenario of the Goddard Institute for Space Studies, GISS, to estimate the increase in groundwater temperature in the native brook trout range, and to estimate the increase in the altitude of the lower stream boundary in a "warmer" climate. The GISS scenario projects a 3.8°C increase in mean annual air temperature for the southern part of the native brook trout range in the next century, which leads to increases of up to 714 m in the altitude of the lower stream boundary, and to significant reductions in area available for brook trout.

Les résultats d'inventaires de ruisseaux à titre d'habitats de l'omble de fontaine (*Salvelinus fontinalis*) révèlent que l'altitude minimum d'un ruisseau à omble de fontaine à la limite méridionale de son aire de répartition endémique augmente graduellement du niveau de la mer à environ 39°12′ de latitude nord, à environ 640 m, à environ 34°40′ de latitude nord. En se basant sur cette limite empirique et un modèle statistique de l'influence de l'altitude et de la latitude sur la température des eaux souterraines, l'auteur formule l'hypothèse que la basse altitude de la limite méridionale de l'aire de répartition endémique est déterminée par l'isotherme de 15 °C des eaux souterraines. Il utilise le scénario de réchauffement du globe élaboré par le Godard Institute for Space Studies (GISS) pour déterminer l'augmentation de la température des eaux souterraines dans l'aire de répartition endémique de l'omble de fontaine et pour déterminer l'augmentation de l'altitude de la limite inférieure en présence d'un climat « plus chaud ». Le scénario du GISS prévoit une augmentation de 3,8°C de la température annuelle moyenne de l'air dans le secteur méridional de l'aire de répartition endémique de l'omble de fontaine au cours du prochain siècle, ce qui mène à une augmentation allant jusqu'à 714 m de l'altitude de la limite inférieure et à une diminution significative du nombre d'habitats disponibles pour l'omble de fontaine.

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The native range of brook trout, *Salvelinus fontinalis* extends from northern Quebec to Georgia. In the northern part of the range, brook trout are distributed from the Atlantic Ocean to Manitoba, whereas, in the south they are confined to the Appalachian ridge. Seguin (1956) and MacCrimmon and Campbell (1969) showed that the native range was delimited in the north by either the Arctic Ocean or the mean January air temperature of about −15°C, in the west by rainfall, and in the south by a mean July air temperature of about 21°C. These summaries of the native range and others (e.g. Bivens et al. 1985) suggest that the minimum altitude of a stream supporting brook trout is higher in the southern part of the range than in the north due to high summer temperatures in the low-altitude streams of the south.

Summer air temperature delimits the southern margin of the native range of brook trout, but is not the most important variable that governs thermal habitat (sensu Magnuson et al. 1979) of brook trout in warm parts of the native range in summer. Groundwater discharge maintains the low temperatures in brook trout streams. (See Power 1980 for a review of the temperature relations of brook trout). Shade by riparian vegetation performs an accessory role because, by reducing insolation, it acts to maximize the cool plumes of groundwater discharge. The temperature of groundwater can be approximated by adding about 1–2°C to local mean annual air temperature (Collins 1925).

There is now a consensus that the climate of the earth will be warmer in the next century, due to the steadily increasing concentrations of CO_2 and other greenhouse gases in the atmosphere (Jaeger 1988; Hengeveld 1990). Recent studies suggest that climatic warming in North America will alter thermal regimes in lakes (e.g. Blumberg and DiToro 1990; McCormick 1990) and shift the distributions of some freshwater fishes northward (e.g. Mandrak 1989; Shuter and Post 1990). The temperature of groundwater is expected to rise with climatic warming (Meisner et al. 1988), which will likely affect the distributions of brook trout in streams.

In an earlier study (Meisner 1990), I estimated that elevated groundwater and summer air temperatures of a "warmer" climate, as implied by the climate warming scenario of the Goddard Institute for Space Studies (GISS; Smith and Tirpak 1989), would shrink summer thermal habitat for brook trout in two southern Ontario streams (325 m, 44°N) by up to 42%. This study suggested that, in groundwater-rich headwater streams,

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elevated groundwater temperature will have a much greater effect on maximum summer stream temperatures than elevated summer air temperatures. Climatic warming should reduce thermal habitat for brook trout in streams at low latitudes and low altitudes throughout this species' native range.

I began the present study with the following objectives: (1) to clarify the inverse relationship between latitude and the lower altitudinal margin of the southern part of the native brook trout range; (2) determine whether the lower altitudinal margin of the native range can be delimited with groundwater temperature; and (3) using the results of (1) and (2), and the GISS climate warming scenario, approximate the potential loss of stream habitat for brook trout in the southern part of the native range, due to climatic warming.

Data Sets

I compiled stream inventories of brook trout habitat from the Ontario Ministry of Natural Resources, New York State Department of Environmental Conservation, Pennsylvania Fish Commission, Maryland Department of Natural Resources, Virginia Commission of Game and Inland Fisheries, and North Carolina Wildlife Resources Commission. Distributional data for Tennessee and Georgia were obtained from England (1979); Bivens (1984); and Fowler (1985). Data for brook trout habitat in West Virginia, Kentucky, and South Carolina were not used in this study.

Each data set consisted of point-in-time samples of stream habitat of brook trout that had been collected during the last 15–40 yr. Sample data varied among states and provinces, but most data included information on the physical, chemical, and biological features of the sample sites as well as current fisheries management strategies for the streams. The variables of interest in this study were site altitude and latitude, and the status of the brook trout population — whether natural or hatchery-supported.

Climate normals were obtained from the Canadian Atmospheric Environment Service and the National Oceanic and Atmospheric Administration of the United States. The climate normal used in this study was the 20–30 yr average of mean annual air temperature at meteorological stations throughout the native brook trout range.

Groundwater temperatures in the native brook trout range were obtained from the United States Geological Survey (USGS). The groundwater data used here were collected during the last 30 yr at a range of altitudes from 35 to 44°N at depths ranging from the surface to 20 m.

The climate warming scenario of the GISS was used in this study. I chose this scenario because its projected change in mean annual air temperature of 3.8°C for the southern Appalachians by the middle of the next century, is intermediate in magnitude to projections of the scenarios of the Geophysical Fluid Dynamics Laboratory, Princeton, and Oregon State University (Smith and Tirpak 1989).

Methods

My objective was to determine if the stream data could delineate a low altitudinal margin of the southern part of the native brook trout range. The first step was to isolate from each data set all site records that did not have brook trout in the species lists, and the site records that were missing fish species lists. From the remaining subset, I selected brook trout sites that were located in the lowest drainage basins of each state and province, and then attempted to sort these sites in descending order of altitude, i.e. headwater sites to main stem sites. From this compiled set, a sample was drawn which consisted of the lowest stream sites of the different drainages. I included some stream sites located at higher altitudes to help delineate the lower stream boundary.

A stream site was included in the analysis only if the site record indicated that the brook trout population was self-reproducing. To standardize the interval of time over which brook trout sites were sampled, I only included sites in the analysis that had been sampled since 1970. In cases where a stream was sampled in more than one location, only the lower site was included.

The stream data for most states (e.g. Georgia, North Carolina, Virginia, Maryland, and New York) did not provide latitude and longitude coordinates for the stream sites. The locations of the sites in these states were obtained from the 7.5-min Quadrangle maps of the United States Geological Survey that most data sets identified for the brook trout sites. When the Quadrangle map for a stream site was not available to me, I used the latitude and longitude coordinates at the centre of the missing map to estimate the location of the stream site. When the Quadrangle maps and altitudes of the stream sites were not identified by a data set (e.g. Maryland), I estimated location and altitude of the sites with either Quadrangle maps or topographic maps of larger scale.

A preliminary lower boundary of brook trout streams from Georgia to Ontario was constructed with the subset of low-altitude brook trout sites. The altitudes of the stream sites in the initial subset of sample records that lacked fish species lists, and that were rated as supporting self-sustained brook trout populations, were compared with the preliminary lower stream boundary — none of these sites were located below the preliminary lower stream boundary.

I estimated the temperature of groundwater at the lower stream boundary with equation (1).

(1) Ground water temperature $= 50.92 - 0.0053$ Altitude $- 0.9330$ Latitude, $N = 112$, $r_1^2 = 0.09$, $r_2^2 = 0.55$, $P < 0.001$.

Equation (1) was obtained by regressing the groundwater temperatures of the USGS against groundwater site altitude and latitude.

The next step was to review the general relationship that states that the temperature of groundwater is about 1–2°C higher than local mean annual air temperature (Collins 1925). This relationship allowed me to use the projected change in mean annual air temperature of the GISS scenario as an estimate of the change in groundwater temperature in a "warmer" climate. I estimated mean annual air temperature at each USGS groundwater site with equation (2).

(2) Mean annual air temperature $= 51.65 - 0.0053$ Altitude $- 0.9920$ Latitude, $N = 453$, $r_1^2 = 0.12$, $r_2^2 = 0.84$, $P < 0.0001$.

Equation (2) was obtained by regressing the long-term mean annual air temperatures at meteorological stations located within and adjacent to the native brook trout range, against station altitude and latitude.

Groundwater temperature in the native brook trout range can be approximated by adding about 1.5°C to local mean annual

air temperature (equation 3), which is consistent with Collins (1925).

(3) Groundwater temperature = 2.15 + 0.940 Mean annual air temperature, $N = 112$, $r^2 = 0.64$, $P < 0.001$.

Equations (1) and (2) estimate a change of 188 m of altitude or about 110 km of latitude for a 1°C change in temperature, which is similar to the general rule that states that a 1°C change in air temperature corresponds to about 170 m of altitude or 80 km of latitude (Peters 1988).

Equations (1)–(3) were estimated from data from the Appalachian Ridge and should not be applied without further testing to other regions. For example, Equations (1) and (2) suggest that groundwater and mean annual air temperatures at sea level at the equator are about 51°C, which is grossly inaccurate.

At the outset of this assessment of the potential effect of climatic warming on the native brook trout range, I made the following assumptions: (1) that the changes in mean annual air temperature at ground level, projected by the GISS warming scenario, apply at all altitudes of the Appalachian ridge; and (2) that the influence of altitude and latitude on groundwater temperature would remain constant in a "warmer" climate.

Results

The minimum altitude of a brook trout stream rises steadily from sea level south of about 39°12′N to approximately 640 m at the southern margin of the native range (Fig. 1)[1]. Each point in Fig. 1 represents one stream site where brook trout have been captured since 1970; no stream is represented more than once. The empirical lower stream boundary (LSB) was fitted by eye. North of about 40°45′N, brook trout streams appear to rise from sea level because data for brook trout habitat for the maritime states and provinces north of Long Island NY were not included in this study.

Groundwater temperature at the empirical lower stream boundary appears to "plateau" as the lower stream boundary rises from sea level south of 39°N (Fig. 1). From about 39–34°N, groundwater temperature only changes by 1°C (14.4–15.4°C), with an average temperature of about 14.8°C. I infer from the relatively constant groundwater temperatures south of 39°N that streams in the native range that receive groundwater at temperatures above about 15°C are too warm in summer to support brook trout. The "plateau" of groundwater temperature also reveals how the effect decreasing latitude (increasing temperature) on brook trout habitat is counteracted by the steady increase in altitude (decreasing temperature), which shows how latitude and altitude interact to maintain low temperatures in brook trout streams. These features of Fig. 1 support my hypothesis that groundwater temperature is the major determinant of the lower boundary of brook trout habitat in the southern part of the native range.

The slight slope of the "plateau" of groundwater temperature south of 39°N implies that brook trout in Georgia are tolerant of higher stream temperatures than brook trout of Northern Virginia and Maryland (i.e. 38–39°N). Because the upper avoidance and lethal temperatures of brook trout in the native range do not appear to vary geographically (McCauley 1958; Power 1980; Hokanson and Biesinger 1989), I judge there is no biological basis for groundwater temperature to vary along the lower stream boundary. The slight increase in groundwater temperature at the lower stream boundary south of 39°N is likely due to the relatively inaccurate method I used to fit it to the brook trout sites.

[1]Complete sets of tabular data (for Fig. 1 and equations (1) and (2) are available, at a nominal charge, from the depository of unpublished data, CISTI, National Research Council of Canada, Ottawa, Ontario K1A 0S2 Canada.

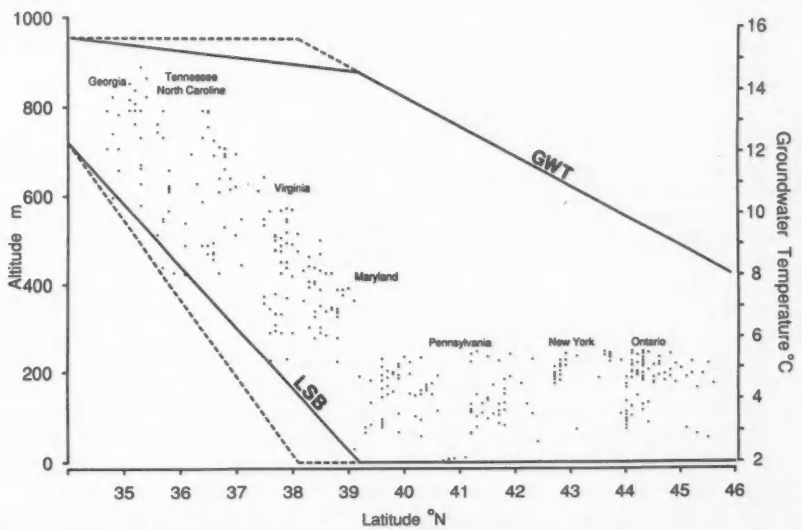

FIG. 1. Scatter plot of altitude of brook trout streams in the southern part of the native range versus latitude. The lower solid line (LSB) that demarcates the empirical lower stream boundary was fitted by eye. The upper solid line (GWT) is groundwater temperature at the empirical lower stream boundary (calculated with equation (1)). The dotted lines represent the theoretical 15.4°C groundwater isotherm and the location of corresponding lower stream boundary; see text.

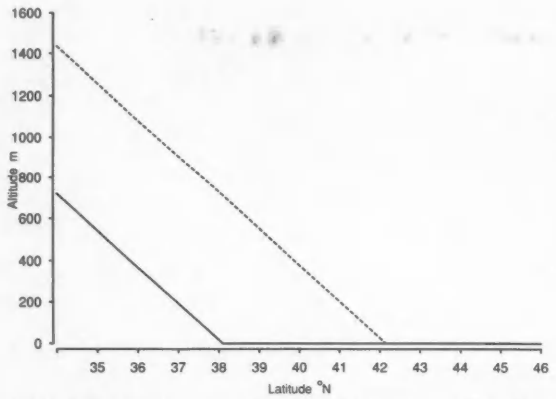

FIG. 2. Theoretical lower boundary of brook trout streams in southern part of native range ———, and lower stream boundary in the GISS climate warming scenario - - - - - -.

I assigned the groundwater temperature of 15.4°C at the empirical lower stream boundary at 34°N, as the theoretical upper groundwater temperature for a brook trout stream. The 15.4°C groundwater isotherm of the Appalachian ridge (and the corresponding lower stream boundary), rises from sea level at about 38°06′N (Fig. 1). I abandoned the first estimate of the upper groundwater isotherm (14.8°C) because the true lower stream boundary that corresponds to it, if plotted on Fig. 1, rises above the lowest stream sites that are south of 36°N. The absence of stream sites near the theoretical lower stream boundary between 36 and 39°N is likely due to cultural stresses. Activities of humans during this century in some parts of the distribution degraded many streams, rendering them unfit for brook trout. Logging operations (e.g. Powers 1929), exploitation, agricultural practices, and pollution have reduced the number of brook trout streams at low altitudes in southern parts of the distribution (e.g. Bivens et al. 1985). Urbanization and pollution likely eradicated stream populations of brook trout in New Jersey and southern Maryland, i.e. 38–40°N (MacCrimmon and Campbell 1969).

Encroachment by introduced rainbow trout, *Oncorhynchus mykiss* in some areas of Georgia and southern Tennessee (e.g. Larson and Moore 1985) has pushed brook trout farther into headwater areas and has excluded these trout from thermal habitat that they otherwise would use. The altitude of the theoretical lower boundary at the southern end of the distribution (~34°40′) in Fig. 1, which is slightly lower than the altitude of the empirical lower boundary, is consistent with the effect of encroachment. Presumably, brook trout would have been found at lower altitudes in these southern areas in the absence of rainbow trout.

Of all the stresses, logging probably had the greatest effect on brook trout habitat in the native range, because in the absence of reforestation, logging permanently increases the temperature of streams and of groundwater (e.g. Brown and Krygier 1970; Hewlett and Fortson 1982).

Impact of Climatic Warming

In a "warmer" climate I infer that the current lower boundary of brook trout streams at low latitudes should shift to higher altitudes. My objective, therefore, was to estimate the increase in the altitude of the theoretical lower stream boundary in the GISS scenario.

The GISS climate warming scenario projects a 3.8°C change in mean annual air temperature at 35–42°N. This projected change in mean annual air temperature was converted to meters of altitude with equation (2) and then added to the theoretical lower stream boundary at each latitude to estimate the altitude of the lower stream boundary in the GISS scenario. North of 38°N, the change in the altitude of the lower stream boundary was calculated from the difference between groundwater temperature in the GISS scenario, as estimated with the projected change in mean annual air temperature, and the theoretical upper groundwater isotherm of 15.4°C. For example, at 37°N, 3.8°C was multiplied by 188 m and added to 171 m which provided an estimate of the altitude of the lower stream boundary at 37°N in the GISS scenario. At 40°N, 3.8 was added to 13.6°C (present groundwater temperature at sea level); from this 15.4°C was subtracted and the difference multiplied by 188 m to estimate the increase in lower stream boundary.

A "warmer" climate, as implied by the GISS scenario, increases the altitude of the theoretical lower stream boundary by up to 714 m (Fig. 2), which reduces area available for brook trout (Fig. 3). The present distribution of brook trout in Fig. 3 was constructed from my data set of low-altitude stream sites and the distribution map of Hendricks (1980). I used the map of Hendricks (1980) to delineate the present distribution in South Carolina, West Virginia, western Pennsylvania, and in the other states and provinces for which I did not have data. My distribution of brook trout in Virginia outlines the distribution of Mohn and Bugas (1980).

The area remaining to brook trout at the lower steam boundary in the GISS scenario was estimated from the contours of 1:1 000 000 scale topographic maps. I sketched the contour of the lower stream boundary on tracing paper from the topographic maps and then photo-reduced the area and superimposed it on the present brook distribution in Fig. 3.

This sketch provides a crude but useful approximation of area at the altitude of the lower stream boundary in the GISS scenario. It does not, however, represent only the areas where brook trout are left in the GISS scenario. I could not construct a plot of the area of brook trout sites remaining above the lower stream boundary in the GISS scenario because I only extracted low-altitude streams from the inventories for the analysis. Also, I did not have all of the data of brook trout habitat in the southern part of the native range (e.g. West Virginia). Thus, the area remaining to brook trout in Fig. 3 represents an estimate of "maximum available area" or "potential habitat" in the GISS climate warming scenario.

The southern part of the native range may become fragmented in a "warmer" climate. Area available to brook trout shrinks in the Blue Ridge Mountains and Allegheny Highlands in northern Virginia, almost disappears in central Virginia, and is restricted to the vicinity of Mount Rogers in southern Virginia. Area available to brook trout disappears in South Carolina and Georgia with climatic warming. Although not included in Fig. 1, the data of Bivens (1984) indicates that the altitudes of the upper boundaries of the brook trout zones in most streams in Tennessee are above the estimated lower stream boundary in the GISS scenario. This suggests that brook trout habitat in that state will be reduced in a "warmer" climate but not eradicated. Some lowland streams in southern areas of Pennsylvania, New York, and in the states northeast of Long Island, NY would become too warm for brook trout.

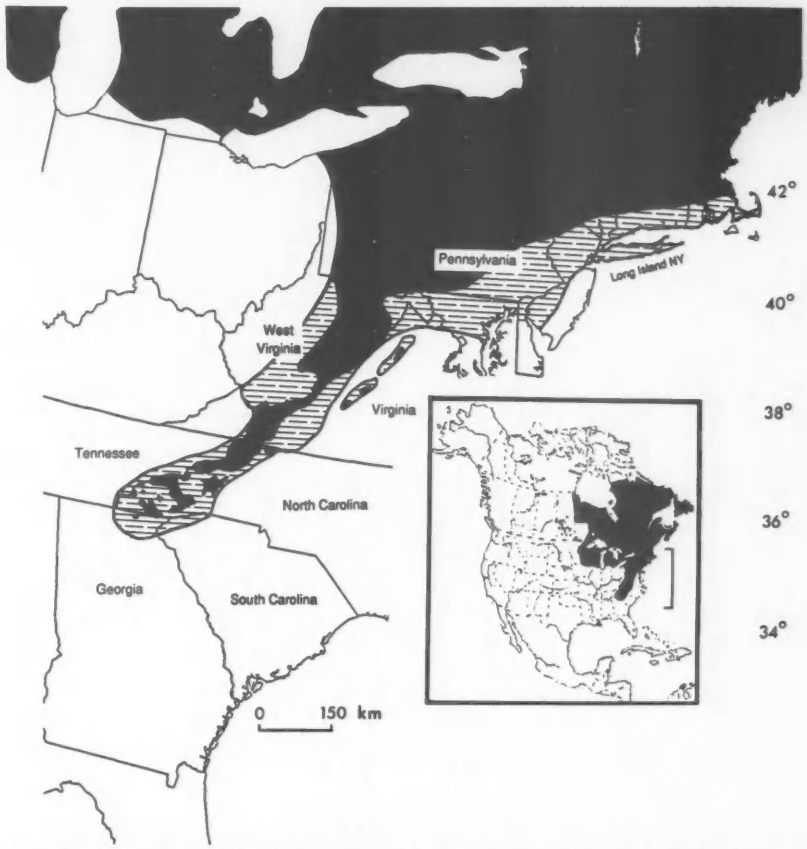

FIG. 3. Present distribution of brook trout in the southern part of the native range (hatched and opaque areas). The present distribution is based on my data and the distribution of Hendricks (1980). The estimated area that becomes unsuitable for brook trout in the GISS climate warming scenario is indicated by the hatched area. Inset shows study area in native range of brook trout (modified from Hendricks 1980).

Discussion

My analysis suggests that undegraded streams in the southern part of the native brook trout range that receive groundwater at temperatures above about 15°C are too warm for brook trout. I infer that groundwater discharge and the riparian forests of these streams cannot maintain enough thermal habitat for brook trout in summer to sustain a population. If groundwater temperatures rise with mean annual air temperatures in climatic warming, thermal habitat of brook trout will likely shrink in streams at low altitudes and low latitudes in summer. However, the reductions in brook trout habitat in this study are drastic, and should be considered only as first-approximations of the direction and magnitude of the effect of climatic warming on the native brook trout range. Further study is needed of the effects of this global phenomenon on lotic environs.

Although not explicit in this study, a factor that directly influences the effect of groundwater on thermal habitat of brook trout in streams is volume of groundwater discharge. The climate warming scenarios project seasonal changes in precipitation in a "warmer" climate (Smith and Tirpak 1989). If summer rainfall changes in the Appalachians, the concomitant changes in baseflow would either exacerbate or alleviate the thermal effects of elevated groundwater temperature on brook trout habitat in summer.

Climatic warming at altitudes and latitudes at which brook trout habitat will not be totally eradicated will likely act as a "predisposing factor" (Manion 1981) to exacerbate the negative effects of past and present activities of man. Rainbow trout will likely take advantage of climatic warming in some streams and push brook trout farther into headwater areas (M. E. Seehorn, U.S. Forest Service, 508 Oak St., Gainsville GA, pers. comm.). The effects of logging activities and pollution on stream populations of brook trout will likely be greater in a "warmer" climate.

Climatic warming should elicit a positive effect on populations of brook trout at high altitudes and high latitudes. Naturalized populations of brook trout in western North America that are constrained by low temperatures (e.g. Hazzard 1933; Scarnecchia and Bergersen 1987) should benefit from higher water temperatures and longer summers. This positive effect may not occur in the native range because stream gradient and natural barriers appear to limit brook at high altitudes (Bivens 1984), not low water temperatures. Thus, stream gradient and

natural barriers may prevent some brook trout populations at high altitudes in the native range from expanding as suitable thermal habitat expands with climatic warming. Populations of brook trout at the northern boundary of the native range should benefit from melting permafrost and longer summers. The potential positive effects of climatic warming on brook trout populations at high altitudes and high latitudes warrant further study.

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Winter Distribution of Bearded Seals (*Erignathus barbatus*) in the Penny Strait Area, Northwest Territories, as Determined by Underwater Vocalizations

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Vocalization surveys conducted in Penny Strait, Northwest Territories, indicated that before ice break-up, bearded seals (*Erignathus barbatus*) preferred regions of less stable ice where break-up occurred early and avoided stable, landfast ice or areas heavily used by walruses (*Odobenus rosmarus*). Water depth did not appear to influence distribution. Numbers of calls increased between mid-April and early June, probably because of an increase in rate of calling by individual seals. Vocalization surveys can be used to separate preferred habitats from unsuitable ones. Using a single hydrophone and our current understanding of bearded seal vocal behaviour, it is not possible to determine the absolute number of bearded seals at or near a site using vocalizations. However, it is possible to measure the relative abundance of seals for spatial and temporal comparisons.

Des études de vocalisation menées dans le détroit de Penny, dans les Territoires du Nord-Ouest, ont révélé que le phoque barbu (*Erignathus barbatus*) préférait, avant la débâcle, fréquenter les régions où la glace était moins stable et où la dislocation des glaces survenait assez tôt, évitant par le fait même la banquette côtière ou les endroits très fréquentés par le morse (*Odobenus rosmarus*). La profondeur de l'eau ne semblait pas influer sur leur distribution. Le nombre de vocalises a augmenté de la mi-avril au début de juin, probablement en raison de l'augmentation du nombre d'appels lancés par les phoques individuels. Les études de vocalisation peuvent servir à déterminer les habitats de prédilection. Étant donné l'utilisation d'un simple hydrophone et notre bagage actuel de connaissances sur le comportement vocal du phoque barbu, il est impossible de déterminer le nombre absolu de phoques barbus présents dans un site ou à proximité. Il est toutefois possible de mesurer l'abondance relative des phoques à des fins de comparaison spatiale et temporelle.

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To date, most attempts to study the distribution and abundance of arctic phocids have been restricted to late spring and early summer when seals haul out on the ice to pup or moult (e.g. Stirling et al. 1982; Kingsley et al. 1985). Visual and photographic surveys are not conducted in winter months. Seals tend not to haul out on the ice then because it is warmer in the water. Inclement weather and darkness further restrict our ability to conduct aerial surveys in winter. The possibility of using underwater vocalizations to census pinnipeds, or at least estimate their relative abundance, has been suggested (Ray 1970; Thomas and DeMaster 1982; Green and Burton 1988). In a preliminary study, Stirling et al. (1983) used the underwater calls of ringed seals (*Phoca hispida*), bearded seals (*Erignathus barbatus*), and Atlantic walruses (*Odobenus rosmarus rosmarus*) to determine their relative abundance at sites in the Canadian High Arctic in late winter and spring.

Bearded seals are circumpolar and boreoarctic in distribution. They prefer areas of moving ice and open water in shallow-bottomed areas, and avoid areas of thick shorefast ice (McLaren 1961; Mansfield 1967; Burns 1970; Davis et al. 1975; Burns and Frost 1979; Stirling et al. 1982; Kingsley et al. 1985). In some areas, bearded seals maintain breathing holes in landfast ice throughout the winter (Mansfield 1967; Stirling and Smith 1977; Smith 1981), probably because the ice in those areas freezes late and breaks up early (Stirling et al. 1983). They also have been observed in waters over 500 m deep during March and April (Finley and Renaud 1980).

During the breeding season, sometime between March and June, male bearded seals produce loud trill-like vocalizations for the purpose of advertising breeding condition, territoriality, or both (Dubrovskii 1937; Chapskii 1938; Ray et al. 1969; Burns 1981; Stirling et al. 1983; Cleator et al. 1989). The objective of this study was to determine the winter distribution of bearded seals in Penny Strait and surrounding waters by recording their underwater vocalizations in April, and to document the pattern of vocalizing between late winter and break-up (in June). We hypothesized that in April fewer vocalizations would be recorded in areas of thick shorefast ice than in areas of open water (i.e. leads and polynyas) or broken ice, and that these habitat preferences would be detected in the vocalization data from year to year. We also expected that the number of vocalizations recorded at all sites would increase between April and

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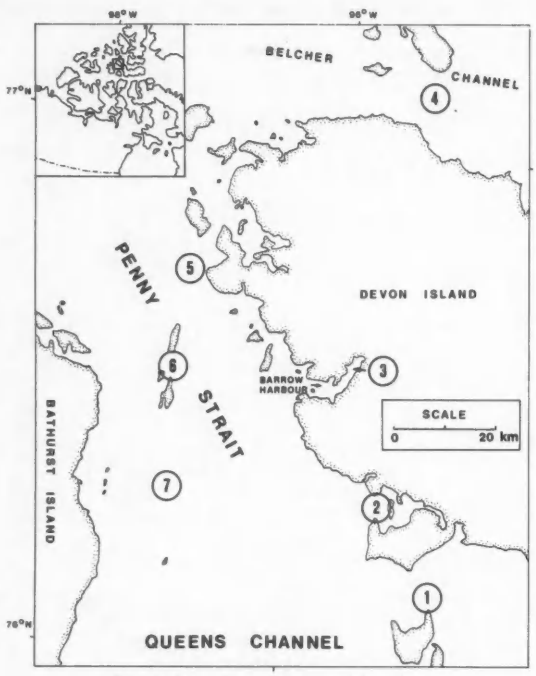

FIG. 1. Locations of the recording sites.

early June as individual males increased their rate of calling in response to progression of the breeding season.

Materials and Methods

Sub-ice recordings were made at seven locations during April–June 1982, and at six of the locations in April 1983 and April 1984 (Fig. 1). As a small percentage (approximately 15%) of bearded seal trills can be heard for distances greater than 20 km from the vocalizing animal (Cleator et al. 1989), the recording stations were located at least 20 km apart, to reduce the likelihood of recording the same seal at two or more sites. Where possible, land masses (usually islands) were used as submarine barriers between sites to dampen or block the transmission of calls from one recording station to another. The recording sites varied with respect to several features: water depth; snow depth; and thickness, stability, age (annual versus multi-year), and type (landfast versus pack) of ice cover (Table 1).

In 1982, sites 1 and 2 were visited on 18 April, 29 April, 4 June, 14 June, and 29 June. The remaining five sites were visited on either three or four of those dates. Some recordings were missed because poor weather precluded reaching the site or because of equipment malfunction. Since few or no vocalizations were heard at site 3 in the spring 1982 surveys, it was not visited in later years. We do not know for certain if the absence of vocalizations at a site means, categorically, that bearded seals are not present or whether they are present but not calling. However, we think it is most likely that the lack of vocalizations at site indicates that bearded seals are not present. In 1983 and 1984, underwater recordings were made on 17 and 26 April and 12 and 25 April, respectively. In the Canadian High Arctic, ice conditions remain relatively stable from December until early May, therefore the distribution of bearded seals in April should reflect their winter distribution.

All underwater recordings were made using a Uher 4200 stereo recorder (frequency response 0.02 to 25.00 kHz +4.5 dB at 19 cm/s) and an International Transducer Corporation 6050C hydrophone (frequency response 0.03 to 75.00 kHz + 1.0 dB, sensitivity −157 dB (re 1 Pa)). During all recordings, the hydrophone was suspended 3–5 m below the underside of the ice. Tape speed was set at 9.5 or 19 cm/s. Recordings were 10 or 20 min in duration.

Daily cycles in rates of bearded seal calling have been reported for the waters around Dundas Island (site 1) in April, with differences in mean hourly rates of calling varying by approximately 300% on a daily basis (Cleator et al. 1989). In order to reduce between-site differences in our study resulting from temporal cycles in calling rates, all vocalization surveys but one were flown between the hours of 0900 and 2000, when mean hourly rates of calling vary up to 1.5 times.

Vocalizations varied greatly in amplitude and that, together with high background noise during some recordings, necessitated a few subjective decisions on whether some faint noises were part of the same call or different ones. In our poorer-quality recordings, there was as much as a 10% difference in the number of calls counted on the same recording in two different listening sessions. For that reason, each recording was played three times and the mean number of calls heard during the three listening bouts was used in analyses.

To determine whether ambient noise levels influenced the numbers of calls counted (e.g. low ambient noise could greatly increase the detection distance of the recording system and hence the number of calls counted), the amplitude of bearded seal calls and ambient noise were measured. Twenty-five recordings and five locations within each of those recordings were randomly selected. At each of the 125 tape locations, spectrum levels were measured at 500 Hz intervals within the approximate frequency range of bearded seals (500–3500 Hz). Spectrum levels were also measured at the start, middle, and end of the bearded seal vocalization recorded nearest the spot on the recording tape at which the ambient noise measurements were made. The mean values calculated for each bearded seal call were divided by the mean values calculated from the corresponding ambient noise measurements to produce a signal-to-noise ratio for each of the 25 selected recordings. These signal-to-noise values were then regressed against their corresponding rates of calling.

To determine whether the vocalization rates were simply a reflection of the amount of area that is was possible to survey at each site rather than an indicator of real differences in the density of vocalizing seals at or near sites, the mean rate of calling at each recording station was divided by the area sampled there. The mean number of vocalizations per minute per 10^6 hectares^{-1} was calculated for each site. A circle with a 20-km radius was drawn on the map around each recording site. It was assumed that the transmission of calls occurred only in straight lines so that areas of water whose sound path to a site was blocked by land were eliminated from the calculation.

Three comparisons were made by means of a Kruskal-Wallace test: among sites in April–June 1982, among sites in April 1982–84, and among years in April 1982–84. As visual inspection of the April 1982–84 data revealed that the calling rates recorded at sites 1 and 2 were noticeably lower than the rates of calling at sites 4–7, the two groups of sites were compared by means of Scheffe's *S*-test on the ranked values to determine

TABLE 1. Habitat information for the recording sites. Snow and ice values represent the means calculated from measurements made in April (1982–84); SD values are shown in brackets. Site 3 was visited only in 1982.

Site number	Lat./Long.	Hydrographic feature	Water depth (m)	Mean snow depth (cm)	Mean ice depth (cm)	Ice age[a]	Character type[b]
1	76°10′N, 94°53′W	Polynya in inter-island channel	25	Variable	Variable	A	L+P
2	76°20′, 95°19′W	Mouth of bay	50	34(21)	170(37)	A	L
3	76°35′N, 95°42′W	Head of harbour	30	3	200	A	L
4	77°05′N, 95°15′W	Inter-island channel	60	9(2)	104(27)	A+M	P
5	76°32′N, 97°09′W	Small inter-island channel	325	9(8)	117(30)	A+M	P
6	76°32′N, 97°09′W	Small inter-island channel	75	8(5)	71(37)	A	L+P
7	76°19′N, 97°02′W	Inter-island channel	260	10(9)	126(37)	A+M	P

[a]A = annual ice, M = multi-year ice.
[b]L = landfast ice, P = pack ice.

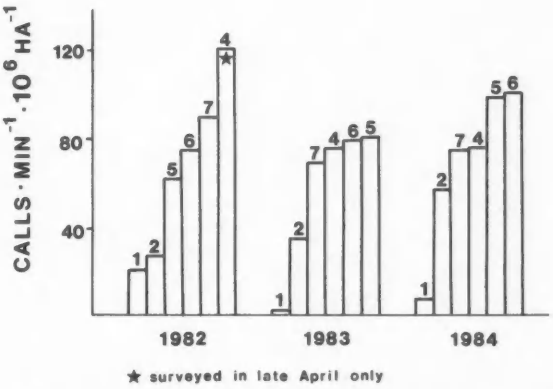

FIG. 2. Mean vocalization rates recorded at each site during the April 1982–84 surveys. Only the area lying within a 20 km line-of-sight radius of each recording site was assumed to be sampled.

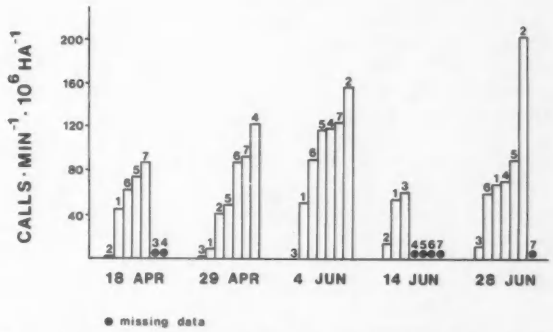

FIG. 3. Mean vocalization rates recorded at each site during the April–June 1982 surveys. Only the area lying within a 20 km line-of-sight radius of each recording site was assumed to be sampled.

if these differences were significant. A critical level of 0.05 was chosen for all tests.

Results

Regression of signal-to-noise ratios against rates of calling for 25 recordings revealed no significance in the relationship between these two variables ($F = 0.03$, $p > 0.87$) thus, ambient noise did not influence the results.

Comparison of calling rates per unit area between sites visited in April 1982–84 revealed significant differences ($H = 19.9$, $df = 5$, $p < 0.005$). Overall, lower rates of vocalization were recorded at sites 1 and 2 than at sites 4–7 ($S = 8.6$, $df = 5.30$, $p < 0.001$) (Fig. 2). The mean numbers of calls recorded in April 1982–84 at sites 1 and 2 were 12.5 calls·min^{-1}·10^{6}ha^{-1} and 37.8 calls·min^{-1}·10^{6}ha^{-1}, respectively. Although three times as many calls were heard at site 2, the calls recorded at site 1 were typically moderate to loud in amplitude, while those at site 2 were usually weaker. Most calls recorded at site 2 probably originated farther offshore in Penny Strait and northern Queens Channel. Sites 4, 5, 6, and 7 had comparable rates of calling in April 1982–84 ($x_4 = 85.8$ calls·min^{-1}·10^{6}ha^{-1}; $x_5 = 80.9$ calls·min^{-1}·10^{6}ha^{-1}; $x_6 = 85.6$ calls·min^{-1}·10^{6}ha^{-1}; $x_7 = 78.5$ calls·min^{-1}·10^{6}ha^{-1}). In spite of the similarity in mean values amongst the four sites, vocalization rates varied considerably between sites and dates: from a low of 42.7 calls·min^{-1}·10^{6}ha^{-1} to a high of 121.8 calls·min^{-1}·10^{6}ha^{-1}. None of the four sites were consistently higher or lower than the rest over the 3-yr period.

Between-year differences in the rates of vocalization in April at the six recording sites were not significant ($H = 0.92$, $df = 2$, $p > 0.05$). The highest mean calling rate was recorded in 1984 ($x = 70.0$ calls·min^{-1}·10^{6}ha^{-1}, SD = 36.4). In 1982 and 1983, the mean rates of calling for the six sites were 61.4 calls·min^{-1}·10^{6}ha^{-1} (SD = 36.6) and 57.1 calls·min^{-1}·10^{6}ha^{-1} (SD = 36.7), respectively.

Significant differences in calling rates were found amongst the seven sites visited between April and June 1982 ($H = 12.2$, $df = 6$, $p < 0.05$). Again, generally fewer vocalizations were recorded at sites 1–3, than at sites 4–7 (Fig. 3). The lowest rates of calling were recorded at site 3 ($x_3 = 18.7$ calls·min^{-1}·10^{6}ha^{-1}) in all of the 1982 surveys except one. On 14 June, five faint calls were recorded there which we suspect probably originated from near the mouth of Barrow Harbour. Below average rates of vocalization were recorded consistently at site 1 in 1982 ($x_1 = 45.4$ calls·min^{-1}·10^{6}ha^{-1}) while at site 2 they varied considerably between surveys ($x_2 = 83.3$ calls·min^{-1}·10^{6}ha^{-1}). For example, between the three June surveys the rate of calling first decreased by a factor of 11 and then increased by a factor of 14. Overall, sites 5 and 6 ($x_5 = 82.8$ calls·min^{-1}·10^{6}ha^{-1}, $x_6 = 75.5$ calls·min^{-1}·10^{6}ha^{-1}), had slightly lower rates of calling than sites 4 and 7 ($x_4 = 104.1$ calls·min^{-1}·10^{6}ha^{-1}, $x_7 = 101.4$ calls·min^{-1}·10^{6}ha^{-1}).

In absolute terms, the number of vocalizations per minute at all sites increased between April and early June 1982, although with the exception of site 2 the rates of calling remained relatively stable amongst the sites during that period (Fig. 3). More

than 100 calls·min^{-1}·10^{6}ha^{-1} were recorded at four of the seven sites on 4 June. Some vocalization rates are missing for sites 4–7 during the mid- and late-June surveys, nevertheless the rates of vocalization decreased at sites 4–6 during the latter half of the month (site 7 was missed in both surveys). In contrast, rates of calling continued to increase at sites 1–3 in June.

Discussion

Our results suggest there was some relationship between rates of vocalization and environmental features. In general, sites 1–3 had lower rates of calling than sites 4–7 in all surveys conducted in 1982–84. Sites 2 and 3 were located in relatively shallow waters covered by thick (>150 cm), stable landfast ice. Low rates of calling were recorded at site 3 during all surveys in 1982 but one (14 June), and most calls recorded at site 2 were weak in amplitude. These data suggest that during the winter and early spring, bearded seals avoid areas such as the inner reaches of inlets, bays, and harbours because of the stable ice conditions that occur there. Previous studies on the habitat preferences of bearded seals gave similar results (McLaren 1962; Mansfield 1967; Burns and Frost 1979; Stirling et al. 1982, 1983; Kingsley et al. 1985).

The five faint calls recorded at site 3 on 14 June 1982 may have originated from the waters surrounding the site, but their weak amplitude suggests they came from near the mouth of Barrow Harbour and travelled along a curved path to reach the head of the harbour. It seems likely that the relatively high rate of calling (60.3 calls·min^{-1}·10^{6}ha^{-1}) for that date resulted from dividing the five calls by an unrealistically small survey area calculated using straight line borders. The geography of the other six sites poses less concern with respect to the 'straight-line' assumption because the ratios of water to land were considerably greater than at site 3.

Bearded seals overwinter most frequently in areas of broken, moving ice over shallow water (<100 m). Areas where the ice contains openings such as polynyas and leads are preferred (Burns and Frost 1979; Smith et al. 1979; Stirling et al. 1981). The polynya at Dundas Island (site 1) persists throughout the winter months. However based on the number of calls recorded there in April, it appears that site 1 is used less by bearded seals than might be expected.

Atlantic walruses also overwinter in the Dundas polynya because of the shallow water and openings in the ice (Stirling et al. 1983). Walruses and bearded seals are both benthic feeders although their preferred foods are different, and bearded seals will consume a wider variety of food items including pelagic fishes such as arctic cod (*Boreogadus saida*) (Vibe 1950; Mansfield 1958; Burns and Frost 1979; Lowry et al. 1980; Smith 1981; Fay 1982; Finley and Evans 1983). Inter-specific competition with walruses for benthic prey may cause seals to move into the deeper waters of Penny Strait and Queens Channel.

Another possible explanation for the apparently low numbers of bearded seals at the Dundas polynya may be that walruses sometimes prey on seals (see reviews by Fay 1960; Krylov 1971). Recent data from the Bering and Chukchi seas have shown that walruses prey upon several species of seals, including bearded seals (Lowry and Fay 1984). Since only young seals were taken, only females with pups and unaccompanied pups or juveniles may be directly affected by the presence of walruses. Indirectly, however, the distribution of adult male bearded seals may be influenced because breeding occurs soon after a female weans her pup (Chapskii 1938; McLaren 1958; Burns 1981). In some cases, females will mate while still lactating (Burns 1981). Breeding males are more likely to vocalize near where females give birth and wean their pups. Consequently, the distribution of adult males may ultimately be affected by the distribution and movements of walruses. Adult bearded seals may also avoid walruses because of the predator avoidance behaviour learned as a pup or juvenile. A similar predator–prey relationship exists in the Antarctic. Leopard seals (*Hydrurga leptonyx*) prey on pup and subadult crabeater seals (*Lobodon carcinophagus*) (Siniff and Bengtson 1977). Adult crabeater seals, although not as vulnerable, still avoid leopard seals perhaps because of avoidance behaviour learned early in life.

Similar rates of calling were recorded at sites 4, 5, 6, and 7 during the 1982–84 surveys. The presence of both first-year and multi-year ice, and the thickness of ice and snow cover were similar between sites 4, 5, and 7 for all years, although water depths at the three sites ranged between 60 and 325 m. Ice conditions at site 6, however, were considerably different. The three islands surrounding site 6 are only 5 km apart; prevailing winds and currents are channelled through the inter-islands waters. Consequently, the annual ice at site 6 was usually thinner (x = 71 cm, SD = 37 cm) than at sites 4, 5, and 7 (x = 116 cm, SD = 31 cm) and consequently, the ice cover there may have tended to break up earlier. Despite differences in water depth, snow thickness, and age, type, and thickness of ice cover between sites 4–7, differences in rates of calling were not statistically significant amongst the four sites.

The 1982–84 vocalization surveys show that during late winter and early spring, bearded seals in Penny Strait and northern Queens Channel avoid waters covered by thick, stable landfast ice which tend to break up late (i.e. like sites 2 and 3). As the winter ice cover does not change appreciably until mid-to late-May in the High Arctic, the results obtained from April surveys probably reflect the distribution of this species throughout the winter (Stirling et al. 1983). With the possible exception of areas heavily used by walruses, bearded seals prefer areas of thinner and less stable ice where break-up occurs early (i.e. like sites 4–7). These results concur with data collected from other areas although some bearded seals have been reported overwintering in stable annual ice, far from open water (Mansfield 1967; Stirling and Smith 1977). Recent field studies in other areas have shown that bearded seals may also overwinter in deeper waters (Mansfield et al. 1975; Finley and Renaud 1980). All of the 37 bearded seals sighted during aerial surveys conducted in the North Water of Baffin Bay in March 1979 were in areas where the average water depth was greater than 200 m and at least 21 (60%) occurred in areas where the water depth exceeded 500 m (Finley and Renaud 1980). In Penny Strait and northern Queens channel, water depths ranged between 75 and 300 m except over shoals near islands. In our study area, bearded seals occurred over a range of water depths.

The mean rates of calling in the Penny Strait area were 1.14 and 1.23 times higher in 1982 and 1984 than in 1983. As calling rates can vary by as much as 1.5 times between the hours of 0900 and 2000 (Cleator et al. 1989), when most surveys were conducted, we do not consider these between-year differences significant.

In 1982, vocalization rates increased between mid-April and early June at most sites. Stirling et al. (1983) also reported a general increase from late winter to early summer at the sites they examined. There are three possible explanations: an

increase in calling rates of individual seals, an increase in the number of seals within range of the hydrophone, or some combination of these. Bearded seals are thought to produce trill vocalizations for the purpose of proclaiming breeding condition, or ownership of territory, or both (Ray et al. 1969). The limited data available indicate that only males produce trills. Male Weddell seals (*Leptonychotes weddelli*) produce a similar trill during the breeding season for advertisement of location (Thomas and Kuechle 1982). It is suspected that individual male Weddell seals increase their rate of vocalization during the breeding season (Green and Burton 1988). Males of that species are polygynous and breed in fast-ice habitat where they defend three-dimensional territories under the ice (Ray 1967; Stirling 1969; Siniff et al. 1974). The mating system of the bearded seal is unknown, although they are probably promiscuous (Cleator et al. 1989). Based on histological evidence, the breeding season for male bearded seals is reported to extend from late winter or early spring to about mid-June (McLaren 1958; Tikhomirov 1966; Burns 1981). In the eastern Canadian arctic waters, between northern Hudson Bay and northern Baffin Island, the peak breeding period occurs in mid-May (McLaren 1958). Males probably call more during the peak of breeding activity than earlier in the breeding season. Because Penny Strait and Queens Channel are surrounded by waters covered with ice types mostly unfavourable for bearded seals, few if any are likely to immigrate from adjacent areas prior to break-up (in June). Thus, the most probable explanation for an increase in calling rates between mid-April and early June is an increase in the rate of calling by individual seals.

With the exceptions of sites 2 and 3, by late June 1982 the calling rates for all sites were similar. Rates of calling for sites 1 and 2 increased from mid-April to late June, whereas rates at sites 4–6 increased and then decreased. Although no surveys were conducted in May, and data are missing for some sites from one or two surveys in mid- and late-June, it appears that the distribution of bearded seals in late spring became less localized than earlier in the year. The change in pattern of calling rates in mid- and late-June probably reflects a change in the behaviour of the seals following the termination of breeding activities. Stirling et al. (1983) also noted the connection between ice break-up and the seals increased freedom of movement and change in vocalization rate.

Underwater vocalizations can be used to determine the distribution and relative abundance of bearded seals between March and June, although more information on the vocal behaviour of this species is required to improve our ability to interpret data obtained by this survey method. Available data suggest that only adult males vocalize; however, the possibility of female vocalization cannot be completely ruled out and should be investigated. Data on the calling rates of individual seals, how these rates may change over time, and how they may be affected by the calls or presence of other individuals are needed. Differences in call amplitude between individuals may exist and individuals may also vary the amplitude of their calls over time. Until it is possible to calculate the locations of individual calling seals, vocalization counts can only be used as a general indicator of distribution and relative abundance in different habitats.

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Phosphorus Deficiency and its Variation among Lakes

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The severity of phosphorus deficiency varies greatly among lakes. Is this variation related to particular morphological, chemical, or biological characteristics of lakes? These questions were addressed by sampling 49 lakes in Quebec, Ontario, and the northeastern United States. The rate constant of orthophosphate uptake (k_1), a measure of orthophosphate demand:supply, proved to be only weakly related to watershed area and to the total phosphorus (P) concentration of the water ($R^2 = 0.210$). Abundance of the biota and other aspects of water chemistry were all unrelated to the intensity of P-deficiency as it varies among lakes. In contrast, within-lake seasonal variability of the rate constant of orthophosphate turnover (k_t, which is approximately equal to k_1) is strongly related to temperature and bacterial abundance ($R^2 = 0.575$ to 0.828). The results indicate that the seasonal variation in phosphorus deficiency depends strongly on bacterioplankton activity, whereas inter-lake variability in phosphorus deficiency is determined mainly by factors other than morphometry, plankton abundance, or trophic richness.

L'importance de la carence en phosphore varie énormément d'un lac à l'autre. Cette variation est-elle liée à des caractères morphologiques, chimiques ou biologiques particuliers des lacs? Cette question a été étudiée au moyen d'échantillons provenant de quarante-neuf lacs du Québec, de l'Ontario et du nord-est des États-Unis. Il a été montré que le taux constant de fixation d'orthophosphate (k_1), mesure de la demande et de l'apport en orthophosphate, n'était que faiblement lié au bassin versant et à la teneur totale en phosphore (P) de l'eau (R^2 = 0,210). L'abondance du biote et d'autres aspects de la chimie de l'eau étaient tous sans rapport avec l'importance de la carence en phosphore puisqu'elle varie d'un lac à l'autre. Par opposition, la variabilité saisonnière du taux constant de renouvellement de l'orthophosphate, dans les limites d'un lac, (k_t qui est à peu près égal à k_1) est fortement liée à la température et à l'abondance des bactéries (R^2 = 0,575 à 0,828). D'après les résultats, la variation saisonnière de la carence en phosphore dépend grandement de l'activité bactérioplanctonique, tandis que la variabilité de cette déficience d'un lac à l'autre est déterminée surtout par des facteurs autres que la morphométrie, l'abondance du plancton ou la richesse trophique.

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Two of the most fundamental characteristics of aquatic systems are the trophic richness and the state of nutrient deficiency of the plankton. The factors that influence trophic richness have been extensively studied, and good predictions can be made regarding the way that trophic status varies from lake to lake (Dillon and Rigler 1974a, b; Vollenweider and Kerekes 1980). The factors that determine the severity of nutrient deficiency of the plankton are less clear. While the growth and production of phytoplankton in temperate zone lakes are commonly limited by phosphorus (Schindler 1977; Wetzel 1983), this is not always true (e.g. Goldman 1972; Goldman 1981), and the severity of phosphorus deficiency varies markedly among lakes (Lean and Pick 1981; Currie et al. 1986; Elsner et al. 1988).

The intensity of nutrient deficiency reflects the ratio of nutrient demand (in moles per litre per time) to nutrient supply ($moles \cdot L^{-1}$). Nutrient demand depends upon the abundance of direct phosphorus consumers — the bacteria and algae — their activity and growth rates and their internal stores of phosphorus (Cemballa et al. 1984). It may also depend upon how actively the community is being grazed (Güde 1986; Wynne and Gophen 1986), the extent to which nutrients are sequestered by zooplankton (Goldman et al. 1987), and other factors. Phosphorus supply depends upon the balance between processes that generate available phosphorus (e.g. external loading, nutrient regeneration by the plankton, etc.), and those that remove available nutrients (sedimentation, adsorption, uptake, etc.).

Although phosphorus deficiency in culture has been extensively studied, remarkably little is known about which of the characteristics of lakes and their plankton discussed above exert measurable influence on the severity of phosphorus (P) deficiency in situ. The most commonly observed indicator of P deficiency is the rate constant of orthophosphate turnover, k_t (defined explicitly below), which has been interpreted as a measure of the ratio of orthophosphate demand to supply (Rigler 1973; Cemballa et al. 1984). Lean and Nalewajko (1979) suggested that k_t is more rapid in small lakes than in large ones, based on comparison of Lakes Ontario and Erie and two small lakes in southern Ontario. Lean (1984) suggested the importance of temperature (although no data were presented), and Lean et al. (1983) proposed that variation in k_t might be related to variation in bacterial abundance.

The present study examines a sample of lakes that differ markedly in the severity of P deficiency in order to determine if particular lake characteristics such as morphology, chemistry, trophic status, etc. are closely related to this variation. By identifying the strongest correlates of deficiency, one suggests the processes that are most likely to control the variation in P defi-

FIG. 1. Location of sampling sites in this study. Thirty-six lakes were sampled; individual points may represent more than one lake. The area northwest of the dotted line is on pre-Cambrian rock on the Canadian shield. The other lakes are located in the St. Lawrence lowlands and the eastern Quebec uplands.

ciency (Rigler 1982). These correlations (obtained using data from many lakes) are then compared with correlations based on the seasonal variation of phosphorus deficiency within two individual lakes (Lakes Ontario and Erie).

The measure of phosphorus deficiency used in this study is k_1, the specific rate constant of orthophosphate uptake. Both k_1 and k_t are defined in the Methods section below. I show that k_t is undefined under some circumstances and that its estimation is statistically problematic. In contrast, k_1 can always be defined unambiguously, and that maximum likelihood estimates of it can be produced easily. Further, k_1 is more clearly the ratio of orthophosphate demand:supply. In most cases, $k_t \approx k_1$ (see Results).

In this study, k_1 is regarded as a measure of the ratio of phosphorus demand to supply for the plankton community as a whole. Although k_1 is determined mainly by bacterial activity (Currie and Kalff 1984a), evidence is provided below that k_1 is also closely correlated with indicators of algal phosphorus deficiency.

Materials and Methods

To investigate the relationships between orthophosphate uptake and lake characteristics, 36 lakes in southern Ontario and Quebec, and northern New York and Vermont were sampled (Fig. 1). These lakes lie on diverse substrates: pre-Cambrian granite, sedimentary rock, glacial outwash, and deep organic soil. They were chosen to vary as much as possible in morphometry, chemistry, and trophic status. Each lake was sampled once between mid-June and mid-August 1986. Small lakes were sampled near their center, larger ones were sampled off-shore.

On site, the depth at the sampling station, Secchi depth, the depth of the epilimnion, conductivity, salinity, and water temperature (with a YSI S-C-T meter) and light extinction (with a Licor quantum meter equipped with a spherical sensor) were measured. Integrated samples were then drawn over the depth of the epilimnion using a length of Tygon tubing. Water samples were returned to a nearby laboratory and analyses were begun within 3 h. In the laboratory, sub-samples were analyzed for chlorophyll *a* concentration, bacterial abundance, total phosphorus concentration, alkalinity, and the orthophosphate uptake rate constant.

Bacterial abundances were determined by direct counts of bacteria stained with DAPI, using a modification of the procedure of Porter and Feig (1980). Blanks were very low and statistically indistinguishable among lakes. Three experimental slides were prepared from each water sample. Three investigators each counted one of the triplicates, and the mean of the three is reported. Bacterial counts were possible for only 23 of the 36 lakes because of methodological difficulties during two sampling expeditions during which the remaining 13 lakes were sampled (staining inexplicably failed and no bacteria were visible on the filters).

Total phosphorus was determined spectrophotometrically following persulfate digestion (Menzel and Corwin 1965). The water samples were not prefiltered to remove larger particles. Chlorophyll *a* concentration was estimated by filtering 250–500 mL of lakewater on Whatman GF/C filters. The chlorophyll was extracted in 90% ethanol and assayed spectophotometrically (Ostrofsky and Rigler 1987). Alkalinity was determined as described by Strickland and Parsons (1972). Dissolved organic carbon was analyzed by stripping water samples of inorganic carbon, oxidizing the remaining organic carbon, and collecting the evolved CO_2 in an alkaline phenolphthalein solution (Ontario Ministry of Environment 1981). Two DOC samples were accidentally lost. All chemical analyses were carried out in quadruplicate.

Lake surface area, mean depth, and watershed area were extracted from published sources or determined from topographical maps. Watershed area was not determined in the case of one riverine lake, and one lake in which two distinct basins were studied.

Estimation of Rate Constants

The orthophosphate turnover constant k_t was originally defined (Zilversmit et al. 1943; Rigler 1964) in terms of a model that postulates that phosphorus is transferred between two states, soluble orthophosphate (S, in $\mu g \cdot L^{-1}$) and particulate phosphorus in the plankton (P, in $\mu g \cdot L^{-1}$) according to first order kinetics:

$$S \underset{k_2}{\overset{k_1}{\rightleftarrows}} P$$

where k_1 is the specific rate of orthophosphate uptake by the plankton (time^{-1}), and k_2 is the specific rate of orthophosphate release from the plankton (time^{-1}). The temporal dynamics of the two compartments are described by the equations:

$$(1) \quad dS/dt = k_2 P - k_1 S$$

$$(2) \quad dP/dt = k_1 S - k_2 P$$

where t is time. Integrating equation (1), it can be shown that the time course of movement of tracer from solution into the plankton is described by the following equation:

$$(3) \quad \frac{S}{T} - \frac{k_2}{(k_1+k_2)} = \left[\frac{S_0}{T} - \frac{k_2}{(k_1+k_2)}\right] \exp[-(k_1+k_2)t]$$

where T is the total amount of phosphorus in the system ($T \equiv S + P$), and S_0 is the soluble activity at time 0. Note that S/T is the proportion of soluble phosphorus, and that at $t = \infty$, S/T comes to an equilibrium level, S_∞/T, which is equal to $k_2/(k_1 + k_2)$.

The rate constant of orthophosphate uptake k_1 is defined as $-(dS/dt)(1/S)$ when all phosphorus is in the form of orthophosphate (equation (1)): i.e. the ratio of the orthophosphate uptake rate by the plankton to the ambient orthophosphate concentration. This represents the ratio of the realized orthophosphate demand (dS/dt) to the orthophosphate supply (S). The orthophosphate turnover constant is defined as $k_t \equiv k_1 + k_2$. The turnover constant thus depends upon both orthophosphate uptake by the plankton and all the processes that regenerate orthophosphate (excretion, grazing, etc.).

Often, orthophosphate uptake data do not conform to this two-compartment model, presumably because orthophosphate exchanges with other forms of phosphorus not specified in the model (Lean 1973). Many alternative models have been proposed to describe multiphasic kinetics (Lean 1973; Lean and Rigler 1974; Norman and Sager 1978); the models postulate additional phosphorus compartments and additional terms in equations (1) and (2). When kinetics do not conform to the two-compartment model presented above, the definition of the turnover constant is unclear. The definition of k_1 as the initial specific orthophosphate uptake rate is unaffected.

There are also statistical difficulties associated with estimation of k_t. Most studies have used graphical "curve-splitting", in which straight lines are fitted to portions of curves in order to estimate single parameters. For example, in some studies S_∞/T is estimated by eye, subtracted from S/T, and the logarithm of the difference fitted as a function of time (e.g. Lean and Rigler 1974). This procedure does not yield maximum likelihood estimates of the parameters, it provides no error estimates, and errors in estimating the first parameter induce bias in estimates of subsequent parameters. Other studies have fitted a straight line to some "initial linear portion" of the curve describing the logarithm of S/T as a function of time (e.g. Lean et al. 1983; Lean et al. 1987a). This procedure is valid only if $S_\infty/T = 0$ (see equation (3)). If $S_\infty/T > 0$, then the latter procedure systematically underestimates k_t by an amount that increases with the period of time over which k_t is estimated. Finally, the logarithmic transformation of S/T prior to regression is invalid. Since the variance of S/T is independent of the mean S/T (present study, data not shown), the logarithmic transformation of S/T introduces heteroscedasticity, which violates one of the assumptions of the regression used to estimate k_t.

In the present study, the rate constants were measured as follows. Triplicate lakewater samples were spiked with approximately 15 $Bq \cdot mL^{-1}$ of carrier-free ^{32}P-orthophosphate. This represents 5×10^{-14} $moles \cdot L^{-1}$ of orthophosphate, which is negligible in comparison to the ambient concentrations of 10^{-10} to 10^{-8} $moles \cdot L^{-1}$ (Currie 1986). The equilibrium cycling of phosphorus is therefore undisturbed by the addition of tracer. Aliquots were withdrawn after 0.33, 1, 2, 4, 8, 16, and 32 min and were filtered on cellulose-ester filters with a 0.22 μm nominal pore diameter. In principle, the appropriate length of incubation depends upon the rate of orthophosphate uptake, which is not known in advance. The geometric series of observations is a compromise that allows reasonably precise estimation of a wide range of values of k_t and k_1. The draw-backs are that, when uptake is very rapid, there are few observations to define k_1, and when uptake is very slow, there are few observations to define $k_2(k_1 + k_2)$. The activity in these aliquots and the total activity in the unfiltered samples were determined by liquid scintillation spectroscopy.

The rate constant of uptake k_1 was determined by fitting a second order polynomial to S/T as a function of t. The polynomial is essentially a Taylor-series approximation of whatever model completely describes the phoshorus dynamics. The rate constant k_1 is given as the tangent to the polynomial at time zero (Currie and Kalff 1984b). Since all the isotope is in the form of orthophosphate at the beginning of the experiment, only the initial rate of uptake is being measured. Thus, the estimate of k_1 is unaffected by any subsequent reactions involving phosphorus in different chemical forms. Earlier work had shown that the estimate of k_1 produced by this method does not depend upon the length of time over which S/T is observed, provided that $S/T \gg S_\infty/T$ (in practice, that $S/T > 0.3$).

In order to compare estimates of k_1 and k_t, the orthophosphate turnover constant k_t was estimated by an iterative nonlinear fit of the two-compartment model (equation (3)), using a quasi-Newtonian algorithm (Wilkinson 1988). S/T was regressed on t, treating $k_2/(k_1 + k_2)$ and $(k_1 + k_2)$ as the parameters to be estimated. For several lakes, there was obvious lack-of-fit in the residuals from the two-compartment model (see Results).

Literature Data

The survey of Currie et al. (1986) of 13 lakes in southwestern Quebec involved some of the same variables observed in the present study. Their data have been incorporated into the present data set and the two sets have been analyzed together. Since they did not measure all of the variables described above, there are missing data in the combined data matrix.

The seasonal variability of phosphorus deficiency within two lakes (Ontario and Erie) was investigated using data compiled from several published studies (Lean et al. 1983; Lean et al. 1987a,b; Pick and Caron 1987; Pick 1987). These synoptic investigations contained observations of the orthophosphate turnover constant k_t alkaline phosphatase activity, Lean and Pick's (1981) phosphorus deficiency index, bacterial abundance, chlorophyll *a* concentration, and temperature as they varied from April to October 1982.

Results

Variation of Phosphorus Deficiency among Lakes

The ranges of conditions observed in the 36 lakes sampled here are summarized in Table 1. Phosphorus deficiency, as estimated by k_1, varied by nearly three orders of magnitude, while total phosphorus and chlorophyll concentrations varied by two orders of magnitude, and bacterial abundance by less than one.

Figure 2 shows a comparison of the estimated values of k_t and k_1 for these lakes. The relationship is tight for low values of the constants, but it degenerates at higher values. This is because the variances of both estimated k_t and k_1 increase approximately with the square of the mean. The most striking outlier (Lake Ontario) represents a case in which the two-compartment model fit the data especially poorly; the variance of the estimated k_t for Lake Ontario is two orders of magnitude greater than that of any other point. The variances of the other observations of k_1 and k_t are generally similar; those of k_t are usually higher, such that the geometric mean variance for k_t is 5.8 times higher than that for k_1.

TABLE 1. The range of conditions encountered in the 36 lakes sampled in Quebec, and Ontario, Canada and New York and Vermont, USA.

Factor	Range	
Temperature	9.1–25.8	(°C)
Conductivity	26–2600	($\mu S \cdot cm^{-2}$)
Light extinction coefficient	0.245–2.40	(m^{-1})
pH	5.61–8.89	
Alkalinity	0.082–3.02	($meq \cdot L^{-1}$)
Total phosphorus concentration	2.7–106.5	($\mu g \cdot L^{-1}$)
Dissolved organic C concentration	1.73–17.4	($mg \cdot L^{-1}$)
Bacterial abundance	2.21–10.07	(10^6 cells$\cdot mL^{-1}$)
Chlorophyll *a*	1.5–74.1	($\mu g \cdot L^{-1}$)
Orthophosphate uptake constant (k_1)	0.008–0.418	(min^{-1})
Epilimnion depth	1.5–9.0	(m)
Mean depth	1.6–54.5	(m)
Surface area	$10.6–1.8 \times 10^6$	(ha)
Volume	$8.8 \times 10^5–1.7 \times 10^{12}$	(m^3)
Watershed area	$166–5.1 \times 10^6$	(ha)
Areal water load	0.832–49.8	(m)
Areal P load from catchment	0.003–8.1	($g \cdot m^{-2} \cdot yr^{-1}$)

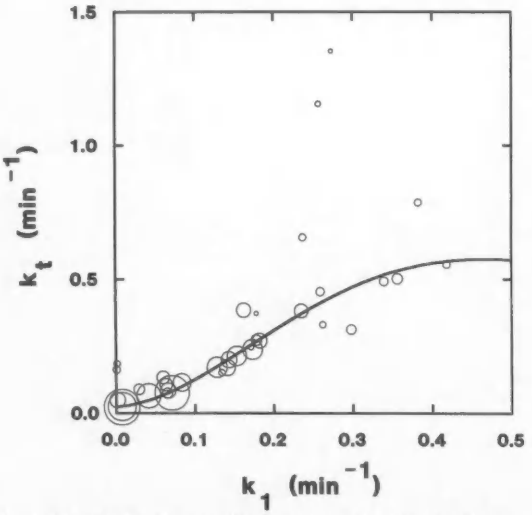

FIG. 2. Relationship between the rate constant of orthophosphate turnover k_t and the specific rate constant of orthophosphate uptake k_1. The units of both constants are time^{-1}. The area of each circle is proportional to $[\text{var}(k_t) + \text{var}(k_x)]^{-2}$, where $\text{var}(k_x)$ is the variance of k_x. Thus, precision increases with the size of the circle: the least precise observation depicted (top center) has a variance nearly four orders of magnitude greater than the most precise observation depicted (lower left). The graph omits one outlier (Lake Ontario, $k_t = 3.40$, $k_1 = 0.00434$) in which the two-compartment model used to estimate k_t fit especially badly. The variance of the Lake Ontario observation was one and a half orders of magnitude higher than that of any other point. The line depicts a fourth-degree polynomial fitted to the observations, weighted according to their variances.

The relationship between k_t and k_1 was explored by first assigning each of the observations a weight proportional to the reciprocal of sum of the variances of k_t and k_1. A polynomial regression of k_t on k_1 yields a fourth-degree polynomial that is slightly sigmoidal in shape and whose slope is close to 1.0 over the entire range of observed k_1 (Fig. 2). Although this regression is not strictly valid (both variables entail error), k_1 statistically accounts for 91.6% of the weighted variation of k_t ($F = 8.5 \times 10^5$, $p < 10^{-5}$). The intercept is slightly, but significantly higher than 0, regardless of the degree of the fitted polynomial. In other words, k_t is slightly higher than k_1, and the two increase roughly proportionally. Biologically, this means that the specific rate of orthophosphate release is usually small and varies little with k_1, so that $k_t \approx k_1$. This is consistent with the observation that, in most lakes, $S_\infty/T \ll 1$, and therefore $k_2 \ll k_1$ (e.g. Rigler 1964; Lean and Rigler 1974; present study). Since k_t is more variable, more difficult to estimate and more difficult to interpret than k_1, and since the two are about equal, only k_1 will be considered further.

Several lake characteristics are significantly related to k_1. The strongest correlations were observed between k_1 and watershed area, lake surface area and the total phosphorus concentration (Fig. 3 and Table 2). There is also a highly significant Spearman rank correlation between k_1 and water temperature (Table 2); however we could not identify a transformation to stabilize the variance of k_1 with respect to temperature (Fig. 3). None of the relationships between k_1 and lake characteristics are particularly strong: the best explains only 18.3% of the variability in k_1. Surprisingly, neither bacterial abundance nor chlorophyll *a* concentration was significantly related to the variation of k_1 among lakes.

The strongest predictor of k_1 is watershed area. This suggests that phosphorus loading from the watershed may be prime determinant of plankton phosphorus status. Phosphorus loading may be estimated using data from published sources: water load may be derived from run-off (Canadian National Committee for the International Hydrological Decade 1978) and watershed area, and phosphorus export from empirical relationships based on land-use patterns (Prairie and Kalff 1986). The annual water and P loads estimated in this manner proved to be less closely correlated with k_1 than was watershed area alone (Table 2).

One may determine if any second variable explains additional variability in k_1 by examining the partial correlation coefficients, holding constant the variables listed above. In particular, one would expect a significant partial correlation with volume or mean depth if the ratio of watershed area to lake size were important. However, after statistically controlling for watershed area, no morphological variable explains significant additional variability. The only variable that does explain further variability is the total phosphorus concentration (partial

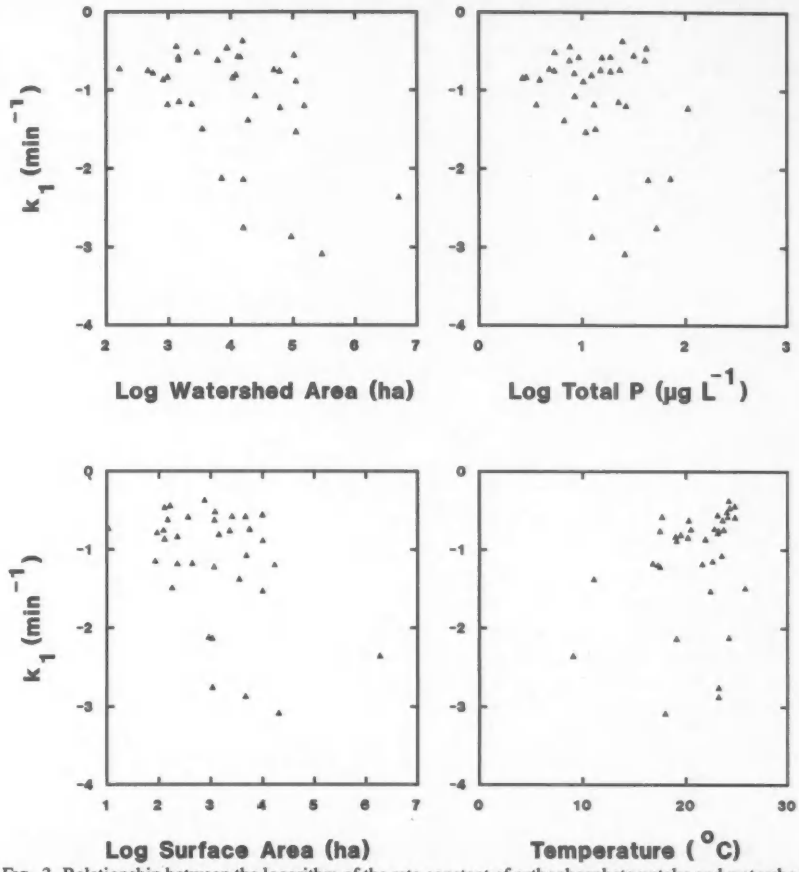

FIG. 3. Relationship between the logarithm of the rate constant of orthophosphate uptake and watershed area (ha), total phosphorus concentration ($\mu g \cdot L^{-1}$), surface area (ha) and temperature (°C) in lakes in Quebec, Ontario, New York, and Vermont.

$r = -0.300$, $n = 43$, $p = 0.05$). The best regression model to predict k_1, based on the present data, is the following:

$$\log(k_1) = 0.454 - 0.263 \log(\text{WSA}) - 0.534 \log(\text{TP})$$
$$\text{adjusted } R^2 = 0.210,\ F = 6.57,\ n = 43,\ p = 0.003$$

where watershed area (WSA) is in hectares and total phosphorus concentration (TP) in micrograms per litre.

Seasonal Variation of Phosphorus Deficiency within Lakes

The seasonal variation of phosphorus deficiency in Lakes Ontario and Erie, as estimated by k_t, was examined using data compiled from the published literature (see Methods). As it varies seasonally, k_t is closely correlated with temperature, chlorophyll, and bacterial abundance in Lake Ontario; in Lake Erie k_t is strongly correlated with temperature and bacterial abundance (Table 3). In both lakes, the relationship is strongest with temperature. After controlling for temperature, bacterial abundance has a significant additional effect only in Lake Erie. The following multiple regressions represent the best predictions of k_t:

Lake Erie: $\log(k_t) = -23.3 + 3.33 \log(\text{BACT}) + 0.078\ T$
adjusted $R^2 = 0.828$, $F = 25.1$, $p < 0.001$, $n = 11$;

Lake Ontario: $\log(k_t) = -2.62 + 0.187\ T$
adjusted $R^2 = 0.575$, $F = 34.8$, $p < 0.001$, $n = 26$;

where bacterial abundance (BACT) is in cells per millilitre and temperature (T) is in degrees Celsius. In contrast to the results obtained among lakes, most of the seasonal variability of P deficiency within lakes can be statistically explained.

Discussion

The first point of this study is that, although the rate constant of orthophosphate uptake k_t has long been regarded as an indicator of phosphorus deficiency, k_1 is a much more appropriate statistic in this regard. k_1 is, by definition, the ratio of orthophosphate uptake to orthophosphate concentration (realized demand:supply); it is defined irrespective of the pathways of subsequent P cycling (whereas k_t may not be), and it can be estimated more precisely and accurately than can k_t. Finally, k_t and k_1 covary closely (except, perhaps, when uptake is rapid and the precision of parameter estimation is poor); they therefore contain similar information.

TABLE 2. Spearman rank correlations (r_s) between the rate constant of orthophosphate uptake k_1 and lake characteristics, and the Pearson parametric correlation (r_p) between log(k_1) and the logarithms of lake characteristics (except temperature, which was not log transformed). The factors from Table 1 that are omitted here were all non-significant at $p>0.50$. To produce these correlations, data were pooled from the present study and from Currie et al. (1986). Unmarked superscript implies $p>0.05$.

Factor	r_s	n	r_p
Watershed area	−0.359[a]	44	−0.428[b]
Total phosphorus concentration	−0.432[b]	47	−0.384[b]
Volume	−0.347[a]	41	−0.338[a]
Surface area	−0.274	49	−0.333[a]
Temperature	0.424[b]	36	0.280
Areal water load	−0.364[a]	43	−0.276
Epilimnion depth	−0.334[a]	36	−0.233
Dissolved organic C concentration	0.362[a]	32	0.140
Chlorophyll *a* concentration	−0.072	49	−0.121
Mean depth	−0.295	41	−0.126
Bacterial abundance	0.132	23	0.085
Areal P load	−0.070	36	0.135

[a] $p<0.05$.
[b] $p<0.01$.

This study makes two main observations. The seasonal variation of k_t within Lakes Ontario and Erie is strongly correlated with temperature and with bacterioplankton abundance. Yet in contrast, the mid-summer variation of k_1 from lake to lake is only weakly related to morphometric and trophic variables. I interpret these observations to mean that most of the seasonal variation in phosphorus deficiency reflects variation in bacterial activity, whereas the among-lake variability in phosphorus deficiency is determined mainly by factors unrelated to plankton abundance, trophic status, morphometry, or the chemical variables observed here.

This conclusion would be unjustified if k_1 or k_t were inadequate indicators of P deficiency. Several lines of evidence suggest that this is not the case. First, Lean et al. (1987a) found that, in seasonal series of samples taken in Lake Ontario, k_t was very strongly correlated with two other indices of phosphorus deficiency: alkaline phosphatase activity (APA) and Lean and Pick's (1981) phosphorus deficiency indicator (PDI). The correlations between APA, PDI, and the present seasonal data from Lakes Ontario and Erie are shown in Table 3. APA, PDI, and k_t are all strongly correlated to one another, and they all correlate similarly with bacterial abundance, chlorophyll *a* concentration, and temperature. Clearly, PDI, APA, and k_t contain similar information regarding phosphorus deficiency.

There is similar evidence regarding k_1 and the variation of phosphorus deficiency among lakes. The data published by Currie et al. (1986) include simultaneous observations of k_1, total alkaline phosphatase activity (APA), total phosphorus concentration (TP), and chlorophyll *a* concentration (Chl *a*). Table 4 shows the correlations among these observations, after logarithmic transformations (to stabilize their variances). APA and k_1 were significantly correlated, and they were more strongly so when APA was expressed per unit chlorophyll or per unit TP. Thus, the variation of k_1 among lakes is also closely related to that of biomass-specific APA, another indicator of P deficiency.

The fact that all the indices of P deficiency are so highly correlated is very significant. APA reflects both algal and bacterial activity (Jones 1972; Currie et al. 1986), PDI is a property specifically of algal activity (Lean and Pick 1981; Lean et al. 1987a), while k_1 and k_t depend mainly upon bacterial activity (Lean and White 1983; Currie and Kalff 1984a; Currie et al. 1986). Bacteria can compete intensely with the phytoplankton for phosphorus in P-limited systems (Rhee 1972; Currie and Kalff 1984c). PDI (the measure of algal deficiency) is significantly correlated with bacterial abundance in both Lake Erie and Lake Ontario, while it is independent of algal abundance in both lakes. This evidence appears to indicate that varying bacterial activity largely determines the seasonal variation in the phosphorus status of the phytoplankton. Presumably, when bacteria take up orthophosphate more quickly, less is left for the algae, which are therefore more deficient. Bacterial abundance, and especially activity, depend strongly upon temperature (Iriberri et al. 1985; Moll and Brahce 1986; Coffin and Sharp 1987), and the present data show that seasonal variation of phosphorus deficiency (by any measure) is strongly related to temperature. Thus, seasonal patterns of phosphorus deficiency of the plankton as a whole probably reflect the varying activity of bacterioplankton and its response to temperature.

The patterns of variation of phosphorus deficiency among lakes are generally consistent with the suggestion made earlier in the literature that the plankton are somewhat more P deficient in smaller, warmer lakes. However, lake size per se is apparently not the critical factor, since watershed area is more strongly related to k_1, and since lake size explains no additional variability after controlling for watershed area. Rather, the plankton are the least P-deficient in eutrophic lakes with large watersheds. Surface run-off in the study area often has a lower

TABLE 3. A summary of the correlations among rate constant of orthophosphate turnover (k_t), the phosphorus deficiency index (PDI) proposed by Lean and Pick (1981), bacterial abundance (bact), chlorophyll *a* concentration (Chl *a*), and temperature (T) as they vary seasonally in Lake Ontario (below the diagonal) and Lake Erie (above the diagonal). Data are taken from Lean et al. (1983; 1987a, b); Pick (1987); and Pick and Caron (1987). The correlations in Lake Erie are based upon 10–15 observations, and those in Lake Ontario on 19–27. n.s. = not significant; n.m. = not measured.

	log(k_t)	log(PDI)	log(APA)	log(bact)	log(Chl *a*)	T
log(k_t)	—	−0.864[b]	n.m.	0.796[b]	n.s.	0.743[b]
log(PDI)	−0.912[b]	—	n.m.	−0.718[b]	n.s.	−0.715[b]
log(APA)	0.860[b]	−0.832[b]	—	n.m.	n.m.	n.m.
log(bact)	0.559[b]	−0.444[a]	0.651[b]	—	n.s.	n.s.
log(Chl *a*)	0.634[b]	n.s.	n.s.	0.511[a]	—	n.s.
T	0.769[b]	−0.527[b]	0.741[b]	0.778[b]	0.658[b]	—

[a] $p<0.05$.
[b] $p<0.01$.

TABLE 4. Pearson correlations among the rate constant of orthophosphate uptake (k_1), alkaline phosphatase activity (APA), total phosphorus concentration (TP), and chlorophyll *a* concentration (Chl *a*) in the data of Currie et al. (1986). All correlations are based upon 14 observations, except those involving TP, for which only 11 observations were available. n.s. = not significant; s.c. = self-correlation.

	log(k_1)	log(APA)	log(TP)	log(chl *a*)
log(APA)	0.627[a]	—	—	—
log(TP)	−0.683[a]	n.s.	—	—
log(chl *a*)	n.s.	n.s.	0.845	—
log(APA/Chl *a*)	0.708[b]	s.c.	−0.834	s.c.
log(APA/TP)	0.855[b]	s.c.	s.c.	−0.825[b]

[a] $p<0.05$.
[b] $p<0.01$.

TN:TP ratio than does the lakewater (Janus and Vollenweider 1981), which should cause lakes with larger watersheds to be less P deficient.

The most striking observation of this study is that so little of the variability in k_1 among lakes can be explained by the variables measured here. This is not due to lack of statistical power: at $n = 36$, correlations explaining as little as 10% of the variability in k_1 can be detected. It is particularly notable that the among-lake variation in phosphorus deficiency was not significantly related to bacterial abundance. Deficiency may be influenced less by bacterial abundance than by bacterial activity, which depends strongly upon temperature. Temperature and k_1 are significantly correlated, but the relationship is not strong. I conclude that mid-summer phosphorus deficiency must be primarily determined by factors other than those studied here.

It is unlikely that, by sampling each lake only once, seasonal effects have confounded the inter-lake comparisons. The among-lake variability of water chemistry and plankton abundance and activity is clearly very much greater than summertime within-lake variability (cf. Table 1 and Lean et al. 1983; Pick and Caron 1987; Lean et al. 1987a; Currie and Kalff 1984a). Moreover, a high percentage of the seasonal variability within a lake is related to temperature and bacterial abundance. If seasonal effects were important in the inter-lake comparisons, then these variables should have shown strong relationships with k_1. Yet they did not. By sampling only during the summer stratified period, seasonal variability was minimized. This indicates that the observed variability of k_1 among lakes is not due simply to seasonal factors.

Rather, some factor or factors that vary among lakes must determine the severity of P deficiency. There are many possibilities. Grazers may strongly influence both P supply and demand in the plankton (Wynne and Gophen 1986; Pengerud et al. 1987). The effects of top predators might also control P availability (Carpenter and Kitchell 1985; Scavia et al. 1986; although this seems unlikely: McQueen et al. 1986; McQueen and Post 1988). It is also possible that phosphorus exchange with the sediments effectively controls nutrient availability to the plankton (e.g. Rigler 1956; Canfield et al. 1984). Rigler (1956) observed strikingly rapid movement of P from the plankton into the sediments and the littoral zone in the small lake that he studied. It is evident that our limited ability to predict phosphorus deficiency represents a major gap in our understanding of the phosphorus dynamics of freshwater communities.

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Comparison of Zygnematacean (*Chlorophyta*) Algae in the Metaphyton of Two Acidic Lakes

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In 1986, Plastic Lake (pH≈5.6), acidified by acidic precipitation, and L.302S (pH≈5.0), experimentally acidified by addition of H_2SO_4, showed similar patterns of unattached benthic growth (metaphyton) of filamentous green algae. The mid to late summer build-up of metaphyton was extensive in both lakes, affecting nearly 100% of the lake perimeters, being concentrated in a shallow band 0.5–1.0 m deep in L.302S and 0.4–2.7 m deep in Plastic Lake. Distribution was negatively related to depth and bottom slope but independent of type of substratum. The areal density of metaphyton in the 0–2 m depth zone of Plastic Lake was estimated to be 1.2 g dry weight (dw) $\cdot m^{-2}$. The productivity of metaphyton standardized by biomass was inversely related to the concentration of algae incubated. Over a common range of incubation concentrations primary production rates were similar in L.302S and Plastic Lake (≈1.6 μg C (mg dw)$^{-1} \cdot h^{-1}$) despite differences in the composition of the communities, predominately *Mougeotia* sp. and *Spirogyra* sp. in L.302S, and *Zygogonium tunetanum* Gauthier-Lievre in Plastic Lake.

En 1986, le lac Plastic (pH≈5,6) acidifié par des précipitations acides et L302S (pH≈5,0), expérimentalement acidifié par l'ajout de H_2SO_4, ont montré des régimes semblables de croissance des algues vertes filamenteuses (métaphyton). L'accumulation de métaphyton du milieu à la fin de l'été était importante dans les deux lacs et touchait presque 100% du périmètre tout en étant concentrée en eau peu profonde variant de 0,5 à 1,0 m dans L302S et de 0,4 à 2,7 m dans le lac Plastic. Sa distribution était en relation négative avec la profondeur et la pente du fond mais était indépendante du type de substrat. La densité aréale du métaphyton dans la zone allant de 0 à 2 m de profondeur dans le lac Plastic a été fixée à 1,2 g dw$\cdot m^{-2}$. La productivité du métaphyton normalisée en fonction de la biomasse était en relation inverse avec la concentration d'algues incubées. En fonction d'un écart commun de concentrations de celles-ci, les taux de production primaire étaient semblables dans L302S et le lac Plastic (1,6 μg C (mg dw)$^{-1} \cdot h^{-1}$) malgré des différences dans la composition des communautés composées surtout de *Mougeotia* sp. et de *Spirogyra* sp. dans L302S et de *Zygogonium tunetanum* Gauthier-Lièvre dans le lac Plastic.

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A characteristic feature of acidified lakes is the proliferation of filamentous green algae (fga) from the family Zygnemataceae in the shallow littoral zone (Grahn et al. 1974; Hendrey and Vertucci 1980; Stokes 1981). There is little doubt that the increased presence of fga in lakes of low pH is related to the acidification process based on the consistency with which the experimental acidification of lakes or lake mesocosms results in the proliferation of fga (Müller 1980; Schindler and Turner 1982; Brezonik et al. 1986; Turner et al. 1987). The habitats of fga in acidic lakes are diverse. Masses of filaments may be attached to benthic substrata, however, the algae can also form loose flocs, mats or clouds that rest on or float just above the lake bottom. It is this latter metaphytic (cf. Wetzel 1983) form which is neither truly attached nor planktonic that is of present interest. The development of metaphyton can be extensive enough to degrade lakes for recreational use (SPR Associates, Inc. 1986), yet the metaphyton has been ignored previously in the calculation of biomass of benthic algae in

TABLE 1. Water chemistry of Plastic Lake and Lake 302S at time of metaphytic sampling. L.302S chemistry data represent the mean of four epilimnetic samples collected weekly between September 2 and 23, 1986. Lake 302S light attenuation data are based on measurements made on September 8 and September 22, 1986. Plastic Lake data are from measurements taken between August 8 and 9, 1986.

	Plastic Lake	L.302S
pH	5.6	5.0
Alkalinity ($\mu eq \cdot L^{-1}$)	0	−5
DIC ($mg\ C \cdot L^{-1}$)	0.20	0.18
Total phosphorus ($\mu g\ P \cdot L^{-1}$)	9.5	9.5
Total nitrogen ($\mu g\ N \cdot L^{-1}$)	200	344
Light extinction coefficient (m^{-1})	0.21	0.65

studies of lake acidification. Similarly, the production of the metaphyton and its contribution to the energetics of the littoral zone are unknown.

The investigation of the energetics of metaphytic algae is potentially complicated by the physical construction of the metaphyton. Within metaphytic growths high densities of algal filaments occur in close association. Variability in both the size of metaphytic aggregates, and the concentration of algal filaments, may affect the biomass-specific productivity of material because of competition for light and nutrients among filaments. Quantitative models developed to predict growth in other benthic algal communities characterized by high densities of filaments have included calculations for density-dependent negative feedback on growth (Fraleigh and Wiegert 1975; Canale and Auer 1982).

As a result little is known about growth regulation of the metaphyton in acidic lakes. Turner et al. (1987) showed that the productivity of epilithic communities dominated by diatoms and bluegreen algae was likely to be reduced by acidification. Epilithic productivity appeared to be regulated by low levels of dissolved inorganic carbon (DIC) in three experimentally acidified lakes. Given that the growth of the metaphyton appears to be enhanced with acidification, it is unlikely that the controls on the growth of the metaphyton will be the same as for the attached periphyton.

In this paper we report on the biomass and productivity of metaphytic algae in acidic lakes. We present our approach for quantitatively sampling metaphytic biomass and investigating the spatial distribution of the metaphyton. We also report preliminary work on the effects of selected factors on the productivity of the metaphyton. Our studies were carried out in two types of acidic lakes in Ontario: L302S, which has been experimentally acidified, and Plastic Lake which has been acidified from anthropogenic acidic deposition (Lazerte and Dillon 1984; Dillon et al. 1987).

Methods

Study Sites

Two acidic lakes with histories of annual development of accumulations of filamentous green algae were studied. Plastic Lake, which has a summer pH of 5.5 to 5.8, develops large areas of green filamentous algal clouds in the littoral zone each summer (M. B. Jackson, pers. comm.). The epilimnetic pH of L.302S was lowered from an initial pH of 6.7 to 6.3 by the addition of sulphuric acid starting in 1982. That year fga developed as a waterline band on rock surfaces in the 0–10 cm depth range (Turner et al. 1987). The following year as the pH was lowered to pH 5.9–6.0, fga proliferated on the bottom to affect 99–100% of the 0–1 m depth zone. With the pH<6.0, attached fga developed extensively in the littoral zone during late summer (Turner et al. 1987). Subsequently, in 1985, detached growths of fga were observed in abundance. The DIC and total phosphorus levels in both lakes at the time of sampling in 1986 were similar (Table 1). Analytical methods for total phosphorus, total nitrogen, and DIC are given by MOE (1981) and Stainton et al. (1977) for results for Plastic Lake and L.302S, respectively. Alkalinity was determined by Gran titration.

Estimation of Biomass for Algal Metaphyton

The distribution of metaphytic biomass in Plastic Lake was examined in detail on August 8–9, 1986, near its maximum seasonal development. The metaphyton was sampled over the 0–2 m depth range, where earlier observations indicated it was concentrated. Ten transects were randomly selected along the lakeshore using perimeter distances. Sampling was stratified using three depth zones: 0–0.49, 0.5–0.99, and 1.0–2.0 m. Five sampling positions were randomly selected from each of the three depth zones in each transect. At each of the 150 sampling positions three measures were recorded: (1) water depth, (2) horizontal distance from shore, and (3) type of substratum. A rectangular box ($0.64 \times 0.41 \times 2$ m; enclosed bottom area 2542 cm^2), open at the top and bottom, was lowered carefully onto the substratum at each sampling position. The bottom 50 cm of the sides of the box were covered by a fine mesh, with an open frame extending to 2 m in height. Floating algal material contained within the area of the box was removed using plastic strainers and placed into plastic bags. Based on visual inspection, removal of the metaphytic algae was effective because the algal material tended to coalesce when manipulated, forming visible clumps.

The volumes of wet samples were measured by settling, and the samples were then frozen. Later, samples were thawed, and oven dried at 60°C for 2 d to determine sample dry weight. Volumes of <1 mL of algae per sample were recorded as zero and these samples were not dried. The practical limit for detection of dry weights was $0.08\ g \cdot m^{-2}$ (average sample wet:dry ratio of approximately 50:1).

Metaphyton sampling variability was described as a function of biomass, i.e. the linear regression of logarithmically transformed variance (s^2) against the logarithmic mean density ($g \cdot m^{-2}$) of estimates from each of the depth zones in each transect was calculated. This relationship was used for two purposes: (1) the slope of the line, or the index of sample aggregation (Taylor 1961), was used to determine the appropriate transformation of biomass data to stabilize the variance; (2) to derive estimates of sample size needed to obtain a desired level of precision given an a priori estimate of biomass (Downing and Anderson 1985).

The shoreline cover of metaphyton in Plastic Lake and L.302S was estimated using a visual survey method described by Turner et al. (1987). The presence of visible metaphyton along the lake perimeter was mapped over the whole lake. Thickness of the metaphyton and the upper and lower depths at which most metaphyton occurred were measured.

Measurement of Primary Production – Plastic Lake

A ^{14}C uptake method for measuring primary production was used in Plastic Lake. Incubations for Plastic Lake were com-

pleted in situ on August 4, 1986. Samples of metaphyton of less than 1 mL wet volume were placed in 300-mL BOD bottles. The bottles were then filled with lake water and incubated at midday for 2 h at a depth of 20–30 cm following the addition of 28 kBq $H^{14}CO_3^-$. This increased the DIC concentration of the incubation bottles by approximately 4%. Each of five individual algal clouds was sampled in triplicate. In addition, two of the five clouds were further sampled in triplicate and incubated at 80% of ambient light intensity to test whether photosynthesis was light saturated. Light intensity was reduced by placing the bottles inside translucent white plastic containers. All incubations were carried out simultaneously. Light flux over the incubation period was measured using an integrating meter (Biospherical Instruments Model QSI-140) over the range of photosynthetically active radiation (PAR) (400–700 nm). Water samples were taken from an area external to the metaphytic clouds for DIC analysis and placed on ice or refrigerated until analysis the next day at the Dorset Research Centre of the Ontario Ministry of Environment (MOE 1981).

Following the incubation period, algal samples were immediately placed on ice in a dark cooler and processed identically within 2 h. The entire sample was blended for 10 s at low speed in a "Waring" blender. Duplicate aliquots of 20 mL of the resulting slurry were filtered onto cellulose acetate filters (0.45 μm) and the filtrate collected in a scintillation vial. The filters were frozen immediately. Filtrates were fixed with Lugol's iodine for later processing. Activity on the filter was determined by burning the material in a Packard TriCarb oxidizer followed by liquid scintillation counting of the resulting sample. The filtrate activity was counted after it had been acidified and bubbled (Shearer et al. 1985) to remove inorganic ^{14}C; sodium thiosulphate was added to clear the Lugol's colour, and Beckman MP fluor was added. Internal standards were used to determine counting efficiencies. The ^{14}C incorporation into organic material during the incubation period was determined by summing the activity in the filtrate and the activity in the filtered material, adjusted for subsampling.

For particulate measurements, additional 50-mL aliquots of the blended slurry were filtered through Gelman A-E glass-fibre filters. Filters were either oven-dried for determination of dry weight or frozen for later determination of chlorophyll *a*. Dimethylformamide extraction (Speziale et al. 1984) was used to determine chlorophyll *a*. The carbon content of metaphyton samples was determined using a Leco CHN analyzer.

The carbon uptake by algae during the incubation period appeared to be linear because the degree of carbon depletion did not affect measured production rates. Production rates for Plastic Lake assays varied widely in relation to the quantity of biomass incubated, yet production rate (standardized by biomass) was independent of the degree of carbon depletion in the incubation chambers ($r^2 = 0.025$).

Measurement of Primary Production – L.302S

Primary production of metaphytic algae in L.302S was measured by adapting inorganic ^{12}C flux techniques developed for periphyton (Turner et al. 1983). Measured volumes of algal suspension were placed in 125-mL glass reagent bottle and filled with lake water. Initial DIC concentrations inside incubation bottles were measured on triplicate 2-mL subsamples. Samples were collected using a glass syringe fitted with a three-way valve and polyethylene fritted tube (Mandel Scientific Co., FL3635) to exclude filamentous algae. Analysis of DIC was by infrared gas analysis (Turner et al. 1987). Bottles were incubated statically in the laboratory for 2 h in an incubator described by Shearer et al. (1985). At the end of an incubation period a second set of DIC samples was immediately collected to determine the change in DIC concentration. Algae in each bottle were filtered onto preweighed GFC filters and dried for determination of dry weight. Aliquots of the metaphyton suspension used in the incubations were filtered on GFC filters for chlorophyll *a* analysis. Samples were frozen and stored in the dark until analysis by high performance liquid chromatography (Kerfoot et al. 1987).

On September 4, 1986, triplicate sets of three different amounts of algal material (0.3, 0.6, 1.7 mL wet volume of algae in 125 mL) were incubated at 750 $\mu E \cdot m^{-2} \cdot s^{-1}$. An additional set of samples (at the intermediate algal density) were incubated at 50% of maximum irradiance. Samples were also incubated in the dark for the determination of dark respiration rates. Similarly, a set of samples was incubated with a two-fold increase in the initial DIC concentration to determine if increased DIC would stimulate photosynthesis. The DIC concentration in the sample bottles was increased by adding 1 mL of a distilled water solution that had been previously bubbled with CO_2 gas.

Further production incubations were carried out on September 24, 1986. Incubations (0.6 mL algal wet volume in 125 mL) were carried out in triplicate at each of five light levels ranging from 0–800 $\mu E \cdot m^{-2} \cdot s^{-1}$. In all incubations, the incubator was maintained within 2°C of the temperature of the epilimnion at sampling time.

TABLE 2. Shoreline development of metaphytic filamentous algae observed during August/September surveys.

Lake	Year	Percent of shoreline covered	Thickness[a] (cm)	Dominant algae (percent of biovolume)
302S	1982	0		
	1984	0		
	1985	38	6	*Mougeotia* (28), *Spirogyra* (59)
	1986	81	11	*Spirogyra* (28), *Mougeotia* (71)
Plastic	1986	97	19	*Zygogonium tunetanum* (97)

[a]Weighted mean for the lake.

Results

Physical Characteristics and Biomass of the Metaphyton

Metaphytic accumulations of fga of varying growth forms (flocs, mats, or clouds) were abundant in the littoral zones of both Plastic Lake and L.302S during the late summer of 1986. They were largely monogeneric growths of Zygnematacean species: 97% *Zygogonium tunetanum* Gauthier-Lièvre in Plastic Lake, and 71% *Mougeotia* sp. in L.302S (this genus may have been *Temnogametum* rather than *Mougeotia*) (Table 2). The previous year in L.302S the metaphyton was dominated by both *Spirogyra* sp. and *Mougeotia* (Table 2). While fga attached to benthic substrata were abundant in L.302S prior to 1985 (Turner et al. 1987), metaphytic accumulations were not observed (Table 2). In addition, the distribution of metaphyton increased substantially between 1985 and 1986 (Table 2). In both lakes the metaphyton was normally first seen in mid-summer,

persisting until its disappearance in mid fall. At the time of this study, the respective growth forms were different in the two lakes. In L.302S, fine bands and mats of varying density covered approximately 81% of the lake perimeter with a mean thickness of 11 cm. The Plastic Lake growths were cohesive aggregates of filaments which formed large balls, cylinders, and irregularly shaped clumps with a mean thickness of 19 cm (Table 2). Essentially the entire perimeter (97%) was covered with metaphyton and the clouds displayed a highly clumped spatial distribution.

The linear regression of sampling variance against fga biomass as dry weight for Plastic Lake was:

$$(1)\quad \log s^2 = 0.02 + 1.44\ (\log B)$$

or

$$(2)\quad s^2 = 1.05\ B^{1.44},$$

where s^2 is sampling variance and B is mean biomass as grams per square metre ($r^2 = 0.81$, $n = 26$). The slope of 1.44 in equation (1) suggests that the sample distribution is aggregated and the data should be fourth-root transformed prior to parametric statistical analysis to remove the dependence of the variance on the mean.

The adequacy of sampling effort given a quadrat size of 0.254 m^2 was examined using the relationship between sampling variance and mean biomass. The number of replicate samples needed for any level of precision can be calculated from equation (1) by combining it with the derivation for the standard error (SE), $SE = (s^2/n)^{1/2}$ (Downing and Anderson 1985). The equation for calculation is:

$$(5)\quad n = 1.05\ B^{-0.56}\ p^{-2},$$

where n is the predicted number of samples required to obtain a level of precision, p, given an estimated mean biomass of B ($g \cdot m^{-2}$). The requisite number of samples for a level of precision of ±20% of the mean ($p = 0.2$) is 24 when $B = 1.2\ g \cdot m^{-2}$ as for the 0–2-m depth range (Table 3). Because we collected 150 samples, the precision of our biomass estimates should be better than 8%.

The wet volume of metaphyton samples was evaluated as a means of collecting biomass data directly in the field. The relationship between algal wet volume and dry weight was linear but with appreciable variability around the regression line, $r^2 = 0.67$, $n = 92$:

$$(6)\quad W = 0.049 + 0.020\ V,$$

where W is algal dry weight in grams and V is algal wet volume in millilitres. While this approach would not be adequate for studies of spatial or temporal dynamics of metaphyton, it would provide a useful means to quickly evaluate the biomass of metaphyton in the field.

The biomass of algal metaphyton varied considerably among the 10 transects around the perimeter of Plastic Lake. Biomass was independent of substratum type but did depend on slope of the lake bottom. Areas of high slope had consistently low biomass (Fig. 1). Biomass was also related to water depth (Table 3). To further evaluate this relationship, samples from the three depth zones of the 10 transects were placed into nine intervals based on the exact sampling depth. Excluding data from the shallowest region, 0–0.2 m where it appears that wave action disperses metaphyton, the relationship between biomass and depth is given by the equation:

$$(3)\quad B^{1/4} = 1.32 - 0.28\ D;$$

where B is biomass ($g \cdot m^{-2}$) and D is depth (m) ($r^2 = -0.91$, n (intervals) = 9). The depth-dependent decline in biomass follows the attenuation of light below 0.2 m (Fig. 2). Based on additional observations made in Plastic Lake, the abundance of metaphyton declined sharply, on average, at 2.7 m. The depth distribution of metaphyton in L.302S was shallower than that observed in Plastic Lake, being most extensive from 0.4–1 m.

Mean biomass of metaphyton was estimated to be 1.2 g $\cdot m^{-2}$ over the 0–2 m depth range in Plastic Lake in early August 1986 (Table 3). Calculated on a whole-lake basis, approximately 43 kg of Zygnematacean algae were present in Plastic Lake in the 3.57 ha of the 0–2-m depth zone.

Production Characteristics of the Metaphyton

The rate of ^{14}C uptake and net photosynthesis of metaphytic algae from Plastic Lake and L.302S, respectively, showed a strong inverse dependence on the concentration of algae incubated. With algal dry weight concentrations in the incubations varying from 0.005–0.1 mg $\cdot$ mL^{-1} in Plastic Lake and 0.038–0.32 mg $\cdot$ mL^{-1} in L.302S, production rates per unit algal dry weight decreased with increasing algal concentration (Fig. 3). The relationship between total production rate of incubation bottles ($\mu g\ C \cdot mL^{-1} \cdot h^{-1}$) and biomass incubated was linear over the ranges of material used but had slopes of less than one. The regression equations were:

$$(7)\quad P = 0.053 + 0.32b$$

$$(8)\quad P = 0.057 + 0.67b,$$

for Plastic Lake ($r^2 = 0.41$, $n = 21$, $p < 0.05$) and L.302S ($r^2 = 0.93$, $n = 15$, $p < 0.05$), respectively (where P is the sample carbon fixation rate ($\mu g\ C \cdot mL^{-1} \cdot h^{-1}$) and b is the concentration of algae incubated in the sample ($mg \cdot mL^{-1}$). An expression for production rate, standardized by biomass, as a function of the quantity of algae incubated is obtained by dividing the right side of equations (7) and (8) by b. After rearrangement, the equation for Plastic Lake is:

$$(9)\quad P_b = (0.053/b) + 0.32$$

and for L.302S is:

$$(10)\quad P_b = (0.057/b) + 0.67$$

TABLE 3. Depth distribution of metaphytic biomass in Plastic Lake on August 8–9, 1986.

Depth (m)	Mean dry weight ($g \cdot m^{-2}$)	Range of dry weights ($g \cdot m^{-2}$)	SE ($n = 50$)	Lake area in depth interval (ha)	Estimates of total dry weight in lake (kg)
0–0.49	1.33	0.2–3.7	0.28	0.91	12.1
0.5–0.99	1.79	0.0–5.8	0.42	0.90	16.1
1.0–2.0	0.82	0.0–2.8	0.18	1.76	14.4
0–2.0	1.19			3.57	42.6

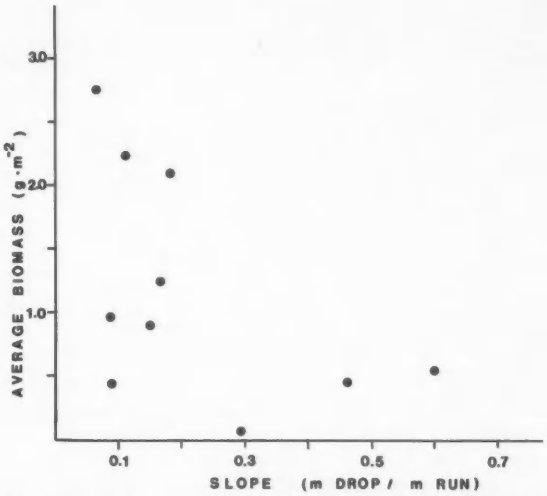

FIG. 1. Metaphytic biomass in Plastic Lake in relation to bottom slope. Average biomass is calculated as the mean of all samples collected over the 0–2-m depth zone for each of the 10 transects. The slope over the 0–2-m depth zone is calculated based on the horizontal distance along the transects to a depth of 2 m.

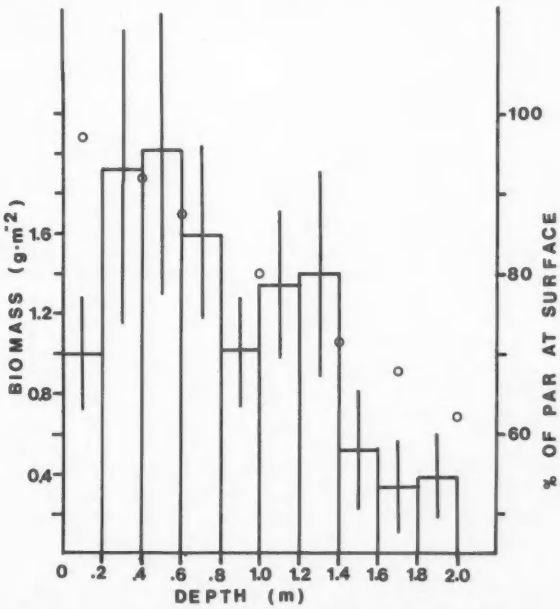

FIG. 2. The depth distribution of metaphytic biomass from 0–2 m in Plastic Lake. Vertical bars represent SE of the mean biomass. Open circle give the percentage of surface PAR at that depth.

where P_b is the production rate ($\mu g\ C \cdot (mg\ dw)^{-1} \cdot h^{-1}$ and b is the quantity of algae incubated ($mg\ dw \cdot mL^{-1}$).

Production estimates from both Plastic Lake and L.302S were similar over a common range of algal concentration, 0.035–0.105 $mg \cdot mL^{-1}$. Over this range, average production rates were 1.5 $\mu g\ C \cdot mg^{-1} \cdot h^{-1}$ for Plastic Lake on August 4 and 1.6 $\mu g\ C \cdot mg^{-1} \cdot h^{-1}$ for L.302S on September 4 (Table 4).

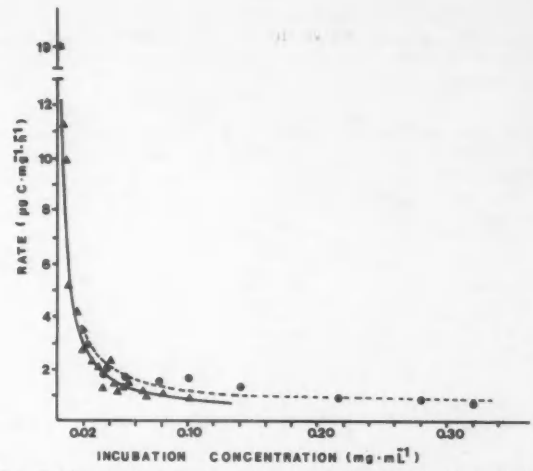

FIG. 3. Metaphytic production rates, standardized by algal dry weight, as a function of the concentration of algae used in the incubation assay. ——— = Plastic Lake; ---- = L.302S. Symbols represent individual observations; curves are derived form equations (9) and (10) (see text).

Such direct comparisons of production rates are subject to three caveats:

(1) the interrelationship of ^{14}C and ^{12}C methods is variable (Turner et al. 1983),
(2) it was unlikely either community was light saturated, and
(3) production estimates were obtained at different times in the season.

The photosynthesis–irradiance relationship of metaphytic algae, as investigated in L.302S revealed that, as expected, production by algae was light dependent. Over the range of 30–800 $\mu E \cdot m^{-2} \cdot s^{-1}$, the algae at an incubation concentration of approximately 0.1–0.2 $mg \cdot mL^{-1}$ were not light saturated (Fig. 4). The compensation irradiance (net photosynthesis = 0) was 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ in late September, but had been substantially lower earlier in the month.

Productivity of the metaphytic algae in L.302S changed between the September 4 and September 24 period. Both net and 'gross' (net photosynthesis adjusted for dark respiration) production decreased over this period. Dark respiration, which was 9% of gross production on September 4, rose to 35% in late September (Table 4). This may indicate mat degradation with the onset of lower temperatures and light levels.

Ambient DIC levels in Plastic Lake and L.302S littoral zones were low, 0.18–0.20 $mg\ C \cdot L^{-1}$ (Table 1). Experimental elevation of DIC levels in solution in the September 4 incubations significantly increased production rates in L.302S (Table 5) in spite of a concurrent decline in pH from 5.2 to 5.0.

Spatial variability in production rates was unimportant in a comparison of five different metaphytic clumps varying in size and location in Plastic Lake. Although there may have been as much as an eight-fold difference in production rates among clumps (Table 6), when variation in sample incubation concentration was eliminated, agreement was within 16%.

Discussion

The extensive accumulation of filamentous green algae (fga) in the shallow littoral zones of Plastic Lake and L.302S

TABLE 4. Production rates of metaphyton from Plastic Lake and L.302S. $P_{gross} = P_{net} + |\text{dark respiration}|$. Production estimates by the carbon-14 method are assumed to be approximately equal to P_{net}. Standard deviations and number of samples in parentheses as appropriate. N/D = no data.

	P_{net}		Dark respiration		P_{gross}	
	($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$)	($\mu g\ C\ (\mu g\ chl\ a)^{-1} \cdot h^{-1}$)	($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$)	($\mu g\ C\ (\mu g\ chl\ a)^{-1} \cdot h^{-1}$)	($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$)	($\mu g\ C\ (\mu g\ chl\ a)^{-1} \cdot h^{-1}$)
L.302S[a]	1.43	1.15	0.14	0.11	1.57	1.26
September 4	(0.23; $n=3$)		(0.02; $n=3$)			
L.302S[b]	1.59	1.27	N/D	N/D	N/D	N/D
September 4	(0.21; $n=6$)					
L.302S[a]	0.82	0.63	0.44	0.34	1.26	0.97
September 24	(0.13; $n=3$)		(0.18; $n=3$)			
Plastic Lake[c]	3.67	0.88	N/D			
August 4	(5.01; $n=15$)	(0.52; $n=15$)				
Plastic Lake[b]	1.45	0.97	N/D			
August 4	(0.40; $n=8$)	(0.55; $n=8$)				

[a]Assays used 0.101 ± 0.003 mg $\cdot$ mL^{-1} for incubations.
[b]Assays used concentrations of algae from .035–0.105 mg $\cdot$ mL^{-1} for incubations; this is a common range of concentrations in L.302S and Plastic Lake.
[c]All assays, excepting those carried out under attenuated irradiance.

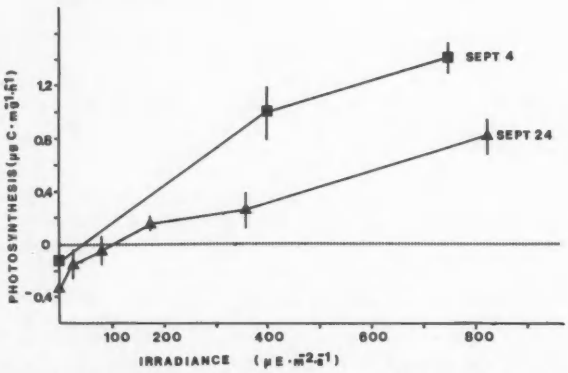

FIG. 4. Photosynthesis–irradiance curves for metaphytic algae from L.302S. Vertical bars represent one SD.

TABLE 5. The effect on photosynthesis of L302S metaphyton of adding dissolved inorganic carbon (DIC) to incubations. Production rates were significantly different ($p<0.05$), t-test.

	P_{net} ($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$) (SD, n)	Inital DIC ($mg\ C \cdot L^{-1}$)
"Ambient"[a]	1.43 (0.32, $n=3$)	0.53
Elevated DIC	2.34 (0.20, $n=3$)	0.91
Percent change	+64	+113

[a]The initial DIC concentration in this treatment was likely higher than in situ (ca. 0.18 mg $\cdot$ L^{-1}) because of respiration of the algal material while held in the dark prior to the experiment.

(Table 2) is typical of many acidic lakes (Stokes 1986). Turner et al. (1987) reported shoreline coverages of up to 100% on rock substrata and 46% on plants in L.223 after experimental acidification. Brezonik et al. (1986) observed 25% coverage of the 0–3-m depth zone of the experimentally acidified north basin of Little Rock Lake. But abundant growth of metaphytic fga from the family Zygnemataceae is not restricted to acidic lakes. It was reported for some circumneutral lakes (Gruendling 1971) and may be characteristic of some types of eutrophic

TABLE 6. Spatial variability in rates of ^{14}C productivity among metaphyton in Plastic Lake, August 4, 1986. Differences in sample rates were largely due to variation in algal density during incubations.

Sample	Observed production rate ($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$) (SD)	Predicted production rate[a] ($\mu g\ C \cdot mg^{-1} \cdot h^{-1}$)	Percent difference
1	2.1 (0.9)	2.2	+5
2	11.1 (8.3)	11.5	+4
3	2.5 (1.5)	2.1	−16
4	1.4 (0.2)	1.3	−7
5	1.4 (0.4)	1.4	0

[a]Prediction calculated from equation (9) (see text).

waters (Hillebrand 1983). We are unaware of any previous estimates of biomass of metaphytic algae in acidic lakes with which our results can be compared. In eutrophic Lake Wingra the mean biomass of mats of *Oedogonium*, a non-Zygnematacean filamentous green alga, was 80 $g \cdot m^{-2}$ (McCracken et al. 1974). Although the metaphytic fga in Plastic Lake was visibly striking, the lake-wide biomass amounted only to 1.2 $g \cdot m^{-2}$ in this oligotrophic lake.

The productivity of metaphytic fga over a common range of incubation concentrations was similar between Plastic Lake and L.302S, 1.5 and 1.6 $\mu g\ C \cdot mg^{-1} \cdot h^{-1}$, respectively, despite compositional differences. It is uncertain whether the similarity in production rates between lakes is due to similar nutrient conditions (Table 1). Although there are no comparable data for metaphyton in acidic lakes, it is instructive to compare our data with the productivity of attached communities which have a high proportion of filamentous green algal species. Müller (1980) measured productivity of periphyton that developed on acrylic plates in lake enclosures that were experimentally acidified. Below pH 6.5, the algal communities were composed predominantly of *Mougeotia* sp. Maximum production rates ranged from 1–6 $\mu g\ C \cdot mg^{-1} \cdot h^{-1}$ in these treatments. Parent et al. (1986) reported a shift to an algal community dominated by *Mougeotia* on tiles in stream channels that were experimentally acidified for 2 mo. The maximum production rate, per unit chlorophyll *a* (chl *a*), of the two acidified treatments was only 0.2 $\mu g\ C \cdot (\mu g\ \text{chl}\ a)^{-1} \cdot h^{-1}$ compared with 0.9 and

1.3 μg C · (μg chl a)$^{-1}$h^{-1} for Plastic Lake and L.302S, respectively. At present there is insufficient information to permit reconciliation of differences in production rates among communities.

Growth of aggregates of metaphytic fga was density-dependent as suggested by the observation that production rates, standardized by biomass, were negatively related to the concentration of algal material used in the incubation assays (Fig. 3). This negative feedback presumably results from a combination of self-shading (light limitation) and reduced rates of nutrient transfer from the water volume to interior algae, thereby increasing nutrient limitation. Thus, the true P_{max} of fga in the absence of this crowding effect is uncertain. But concentration of fga used in these incubations was small compared with the actual concentration occurring in metaphytic aggregates in the lakes. If we assume that the results of our incubation assays accurately describe metaphytic algae growing in situ, then we expect that the rate of accrual of metaphytic biomass in Plastic Lake and L.302S is strongly influenced by a density-dependent negative feedback on growth. As aggregations of filaments grow in size, there is a progressive reduction of the surface area to volume ratio of the aggregate. Conceivably, quiescent areas could surround internal filaments leading to increased thickness of boundary layers enveloping these cells. Depletion or retardation of movement of materials is likely to occur as they pass through the filament mass. An example of such a depletion was observed for DIC. Inside *Mougeotia* clumps in L.302S at noon on a sunny day, DIC concentrations were as low as 0.04–0.05 mg C · L^{-1} compared with 0.2 mg C · L^{-1} in water just above the clump. Despite the likelihood of the negative effects of increasing size on growth rate, the metaphytic form may also enhance the growth potential of the community by facilitating efficient recycling of nutrients within metaphytic aggregates. Materials lost by interior algae due to respiration, leakage, and senescence may be recycled within the community and only slowly lost from the filament mass.

Turner et al. (1987) suggested that the availability of DIC regulates photosynthesis of some benthic algae in acidic lakes. Concentrations of DIC in acidic water, pH <6, are low due to the loss of HCO_3^- combined with the low concentration of CO_2 in water open to the atmosphere (Stumm and Morgan 1981). The apparent carbon limitation of the metaphyton in L.302S, as inferred from stimulation of photosynthesis by experimental additions of CO_2 (Table 5), agrees with results from other benthic algal communities in L.302S. The epilithic algal community had higher production rates when DIC levels in incubation chambers were elevated experimentally (Turner et al. 1987).

Photosynthesis of metaphytic material from L.302S was light limited at irradiances up to 800 μE · m^{-2} · s^{-1}, the highest level examined. These results do not give absolute light requirements for algal cells, but indicate the requirements for a specific concentration of cells at a particular level of self-shading. Because the incubation concentration (0.1–0.2 mg · mL^{-1}) was low compared with the density of a formed algal mass in L.302S (approximately 0.4–0.8 mg · mL^{-1}) we expect self-shading to be important under natural conditions. The negative depth distribution of biomass observed in Plastic Lake from 0.2 to 2 m also suggested that light limited development of metaphyton. Lesser amounts of metaphyton in the 0–20-cm depth zone is consistent with the observation by Gruendling (1971) that metaphytic algae in Marion Lake, previously stained with crystal violet, were dispersed at 0.5 m depth by water movement, whereas clumps of algae at 1.0 and 2.0 m were unmoved.

The depth–metaphyton biomass relationship observed in Plastic Lake may also be related to other factors. The degree of water movement decreases, and the hydrostatic pressure increases, over the 0–2 m zone. Water movement may enhance metaphyton development by increasing the supply of nutrients. Increased supply may occur because of either reduced boundary layers around cells and enhanced diffusion, or because of increased exchange of periphytic nutrients with the overlying water. Low hydrostatic pressure in the shallows may also enhance metaphyton development by facilitating oxygen bubble growth. Hillebrand (1983) demonstrated that bubble formation was important for the buoyancy of metaphyton in shallow ditchs and ponds in the Netherlands. The ease of oxygen bubble growth decreases strongly over the 0–2-m depth range (Ramsey 1962).

It is doubtful that the increased water clarity associated with anthropogenic acidification (Yan 1983) as observed in L.302S (Shearer and DeBruyn 1986) is sufficient as a stimulus for increased development of metaphyton. Yet increased transparency over periods of active growth of the metaphyton should enhance biomass accrual and development with depth.

The metaphyton community in acidic lakes may be an important component in the algal production of the littoral zone. A preliminary estimate of the areal production rate of metaphyton for Plastic Lake in August 1986 was calculated by multiplying the mean biomass per unit area by the mean production rate measured per unit biomass. Corresponding production rates of the periphyton complex associated with *Eriocaulon septangulare*, an important substratum in the littoral zone of Plastic Lake, were measured the following day (E. T. Howell, unpubl. data). The metaphyton was as productive as the attached community on the basis of littoral zone area, with rates of 5.2 and 4.7 mg C · m^{-2} · h^{-1}, respectively. But biomass of the metaphyton was one-tenth that of the epiphyton, 5.9 mg chl *a* · m^{-2} and 60 mg chl *a* · m^{-2} (SD = 16.3, n = 12), respectively. Hence, the chl *a*-normalized production rate of metaphyton was 10 times higher than that of the attached community, 0.88 μg C · (μg chl *a*)$^{-1}$ · h^{-1} compared with 0.078 μg C · (μg chl *a*)$^{-1}$ · h^{-1} (SD = 0.025, n = 4), respectively.

Accurate and precise measurements of the productivity of the metaphyton are presently difficult to make. The problems are firstly, to determine areal biomass of a highly aggregated community and secondly, to relate areal biomass to specific productivity in a way that considers density effects in order to give an estimate of areal production. Methods utilizing oxygen microprofiles are effective for measuring areal production rates of benthic communities with high algal densities (Revsbeck and Jorgensen 1981). However, these methods are less useful for the clumps of metaphyton which are physically unstable. Because metaphytic accumulations are annual it may be simplest to estimate net productivity (adjusted for herbivory and other loss processes) by estimating maximum seasonal biomass.

Further work on the metaphyton of acidic lakes is required to determine both the causes and the consequences of metaphyton development. There may be differences in the relative abilities of benthic algal communities to photosynthesize under low DIC concentrations that may account for the shifts in the apparent success of the communities between acidic and circumneutral lakes. Additional information on the patterns of consumption of fga in acidic and circumneutral is required to

evaluate the role of herbivores in the success of fga and the metaphyton.

The consequences of metaphyton development for the littoral zone of acidic lakes are possibly extensive. The metaphyton constitutes a new habitat distinct from that usually associated with the littoral zone in circumneutral lakes; it is likely to result in diversification of the littoral food web and is an additional source of autotrophic production. However, the success of the metaphyton may be at the expense of the development and productivity of other communities in the littoral zone. At present it is uncertain whether the development of metaphyton in acidic lakes enhances or impedes energy production and processing in the littoral zone.

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Allozyme and Mitochondrial DNA Variation among Three Species of *Stizostedion* (Percidae): Phylogenetic and Zoogeographical Implications

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The percid genus *Stizostedion* consists of five species, three in Europe and two in North America, and is believed to have evolved in Europe and then later colonized North America. Three hypotheses exist concerning the route and timing of this colonization: a Miocene Atlantic "riverine" route; a Pliocene Beringian route; and a late-Pleistocene Atlantic route. To test these hypotheses, allozyme and mitochondrial DNA variation were compared in three *Stizostedion* species, *S. canadense* and *S. vitreum* from North America, and *S. lucioperca* from Europe, to determine their genetic relatedness. Divergence times between *S. canadense* and *S. vitreum* were estimated as 3.12 ± 1.33 million years before present (MYBP) from allozyme data and 4.06 ± 0.73 MYBP for mtDNA data, while divergence times between the North American species and *S. lucioperca* were 10.59 ± 2.74 MYBP and 7.86 ± 1.18 MYBP, respectively. These results are concordant with the hypothesis that colonization of North America by *Stizostedion* occurred during the Pliocene via Beringia.

Le genre percidé *Stizostedion*, qui comprend cinq espèces, dont trois en Europe et deux en Amérique du Nord, a évolué, croit-on, d'abord en Europe pour ne coloniser l'amérique du Nord que par la suite. Il existe trois hypothèses sur le trajet et la chronologie de cette colonisation : un trajet « riverain » dans l'Atlantique au Miocène; un trajet « beringien » au Pliocène; et un trajet atlantique au Pléistocène supérieur. Pour vérifier ces trois hypothèses, les variations des allozymes et de l'ADN mitochondrial ont été comparées chez trois espèces de *Stizostedion*, soit *S. canadense* et *S. vitreum* en Amérique du Nord et *S. lucioperca* en Europe, afin de déterminer leurs relations génétiques. Le temps de divergence entre *S. canadense* et *S. vitreum* a été évalué à 3.12 ± 1.33 millions d'années avant aujourd'hui, d'après les données sur les allozymes et à 4.06 ± 0.73 millions d'années à partir des données sur l'ADNmt, tandis que le temps de divergence entre les espèces nord-américaines et *S. lucioperca* s'élevait respectivement à 10.59 ± 2.74 millions d'années avant aujourd'hui et 7.86 ± 1.18 millions d'années. Ces résultats concordent avec l'hypothèse selon laquelle la colonisation de l'Amérique du Nord par *Stizostedion* a eu lieu pendant le Pliocène, via Beringia.

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A number of workers have employed sequence variation in animal mitochondrial DNA (mtDNA) to examine the genetic relatedness and systematics of closely related species (Wilson et al. 1985; Avise 1986; Avise et al. 1987; Moritz et al. 1987). Allozyme variation has also been used in a similar manner and for a longer period (e.g. Oxford and Rollinson 1983). However, few workers have attempted to compare allozyme and mtDNA data sets, and most of their studies have dealt with interspecific hybridization and introgression (Baverstock et al. 1983; Ferris et al. 1983; Avise and Saunders 1984; Avise et al. 1984; Szymura et al. 1985; Lamb and Avise 1986, 1987; Edwards and Skibinski 1987; Gyllensten and Wilson 1987a; Harrison et al. 1987; Billington et al. 1988). With the exception of studies on the higher primates (e.g. Nei et al. 1985), few have compared the extent of allozyme and mtDNA divergence between species (Avise et al. 1979a,b; Lansman et al. 1983; Kessler and Avise 1985; DeSalle et al. 1987; Becker et al. 1988; Tegelström et al. 1988; Schweigert and Withler 1989).

Tegelström et al. (1988) found a high correlation between Nei's distance (*D*) values (calculated from allozyme data) and the percent sequence divergence of mtDNA (*p*) between pairs of closely related species, mainly small mammals, indicating that differences in mtDNA among taxa are not random when compared with divergence in protein coding nuclear genes. This survey did not include data on fish species and such data are relatively scarce, however, a recent comparison of allozyme (Grant et al. 1988) and mtDNA divergence (Becker et al. 1988) between two marine fish species did show concordance between the two data sets. Furthermore, Kessler and Avise (1985), in a comparison of avian and nonavian taxa (including the fish genus *Lepomis*), found a strong positive, yet curvilinear relationship between mtDNA sequence divergence (*p*) and Nei's *D*; this relationship was approximately linear for *p* and *D* values of up to 15% and 0.30, respectively.

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Here we compare mtDNA and allozyme variation in three species of the freshwater fish genus *Stizostedion* (Percidae). *Stizostedion*, the pikeperches, comprises five species, three of which are found in Europe (zander, *S. lucioperca* (L.); Volga zander, *S. volgense* (Gmelin); sea pikeperch, *S. marinum* (Cuvier)) and two in North America (walleye, *S. vitreum* (Mitchill); sauger, *S. canadense* (Smith)) (Collette and Banarescu 1977; Craig 1987). A further taxon, the blue walleye (*S. vitreum glaucum* Hubbs), was found in Lake Erie and Lake Ontario, but became extinct in the late 1960's (McAllister et al. 1985). The distribution of these species is described in detail by Collette and Banarescu (1977) and their biology is reviewed by Craig (1987).

It is generally accepted that *Stizostedion* originated in Europe and later colonized North America (Svetovidov and Dorofeeva 1963; Collette and Banarescu 1977; Craig 1987), but there is some controversy over when this colonization took place. Fossil records of *Stizostedion lucioperca* have been found in Siberia dating to the Pliocene (Yakovlev 1961 — cited by Svetovidov and Dorofeeva 1963). However, the earliest fossil records of *Stizostedion* from North America date from the late-Pleistocene (Smith 1981; Cavender 1986) and most workers consider that *Stizostedion* colonized North America during the Neogene (probably Pliocene) through Beringia (Svetovidov and Dorofeeva 1963; Collette and Banarescu 1977). However, Cihar (1975), in a study of the genus *Perca*, questioned the evidence for a Beringian colonization route for both *Perca* and *Stizostedion* and instead proposed that both genera colonized North America by an Atlantic route during the late-Pleistocene, utilizing the brackish water along the margins of the retreating ice sheets. Other workers have suggested that *Stizostedion* colonized North America by a North Atlantic riverine connection during the Miocene (Lindberg 1961, 1962 — cited in Svetovidov and Dorofeeva 1963) or earlier (Balon et al. 1977).

In this paper we examine mtDNA and allozyme divergence among the two North American species and one of the European species (*S. lucioperca*) to determine their relatedness and possible divergence times with respect to the above colonization hypotheses.

Materials and Methods

Collections

One hundred and sixty-two walleye were collected from 10 sites in the Great Lakes watershed between April 1985 and April 1987 (see Billington and Hebert (1988), for details). Four sauger were also collected, three from the Great Lakes and one from northern Manitoba (Billington et al. 1988). Fish were returned to the laboratory, on ice, where 5 g of liver were used to prepare mtDNA samples, while the remainder, together with the head, was frozen at −40°C for subsequent allozyme analysis.

Zander were collected from the River Ouse, near St. Ives, Cambridgeshire, England, in December 1985. Livers from three fish were used to prepare mtDNA. Heads and livers from two additional zander were frozen for subsequent allozyme analysis.

Allozyme Analyses

Small pieces of white muscle, eye and liver were individually homogenised with a few drops of 0.2 M Tris-HCl, pH 8.0. The crude homogenate was centrifuged for 1 min at 10 000 × g and the supernatants retained for electrophoresis. Two types of electrophoresis were carried out: horizontal starch (12.5% gels, Connaught starch) and cellulose acetate (Titan III gels, Helena Laboratories). Two buffer systems were employed for the starch gel electrophoresis: continuous Tris-citrate (electrode buffer 0.25 M Tris, 0.057 M citric acid, pH 8.0; gel buffer 1:25 dilution of electrode buffer; 200 V for 4 h) and discontinuous Tris-citrate (electrode buffer 0.30 M borate, pH 8.2; gel buffer 0.076 M Tris, 0.005 M citric acid, pH 8.7; 300 V for 4 h). A single buffer system was used for the cellulose acetate gels: Tris-glycine (gel and electrode 0.025 M Tris, 0.192 M glycine, pH 8.5; 200 V for 15 min). Twenty-three enzyme systems were screened using these electrophoretic systems (Table 1). The substrate used for esterase was α-naphthyl acetate. Stain recipes were modifications of those given by Shaw and Prasad (1970) and Harris and Hopkinson (1976). The two electrophoresis methods yielded concordant results, except that the starch system differentiated between the *Ldh-1* of the zander and that of walleye/sauger, whereas the cellulose acetate system failed to separate these allozymes.

The most frequent allele at each locus in the walleye was designated 100; other alleles were numbered according to the relative mobility of their product to that of allele 100. Where multiple loci code for enzymes, loci were numbered in order of increasing anodal mobility of their products.

Mitochondrial DNA Analysis

Mitochondrial DNA was isolated and digested using standard procedures (Billington and Hebert 1988). Twenty-four hexanucleotide sequence recognition endonucleases wee used: *Apa* I, *Ava* I, *Bam* HI, *Bcl* I, *Bgl* II, *Bst* EII, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Kpn* I, *Mlu* I, *Nco* I, *Pst* I, *Pvu* I, *Pvu* II, *Sal* I, *Sca* I, *Sma* I, *Sst* I, *Sst* II, *Xba* I, and *Xho* I. An incubation period of 4 h was usually sufficient to achieve complete digestion of 10 ng of *Stizostedion* mtDNA in 15 μL of digestion mixture, but *Ava* I and *Hind* III required 12–16 h to complete digestion. The resulting fragments were end-labelled with ^{32}P, separated by electrophoresis (using 1.0% agarose and 4.0% polyacrylamide gels), vizualized by autoradiography, and sized as described by Billington and Hebert (1988).

Restriction endonuclease cleavage site maps were constructed by conducting an extensive series of single and double digests (Maniatis et al. 1982). Double digests, using an endonuclease that produces a blunt-ended cut along with one that produces a sticky-ended cut, greatly simplified the mapping procedure, because fragments produced by each type of enzyme could be more easily identified (N. Billington, P. D. N. Hebert and R. D. Ward, pers. observ.). In this manner, the relative ordering of the cut sites with respect to each other was determined. Usually, samples of mtDNA from each of the three species, cut with a particular endonuclease, or pair of endonucleases, were run in adjacent lanes on a gel, to facilitate scoring co-migrant fragments. The three mtDNA restriction maps, so produced, were lined up against each other using four conserved *Hind* III sites, three conserved *Sst* II sites, and a conserved *Sma* I site.

Results

Allozyme Data

Allele frequencies at each locus were determined for each *Stizostedion* species (Table 2). For walleye, these frequencies are the weighted averages across the populations assayed for that enzyme; note that not all populations were screened for

TABLE 1. Proteins investigated in walleye, sauger, and zander. Starch buffer: C = continuous tris-citrate; D = discontinuous tris-citrate. Cellulose acetate: good = clear, sharp bands; fair = slightly fuzzy bands but of acceptable definition; poor = inadequate resolution, N.D. = not determined.

Protein	E. C. number	Starch buffer	Cellulose acetate
Alcohol dehydrogenase (*Adh*)	1.1.1.1	D	Good
α-glycerophosphate dehydrogenase (α*Gpdh*)	1.1.1.8	C	Good
Sorbitol dehydrogenase (*Sdh*)	1.1.1.14	D	Good
Lactate dehydrogenase (*Ldh*)	1.1.1.27	C	Good
Malate dehydrogenase (*Mdh*)	1.1.1.37	C	Poor
Malic enzyme (*Me*)	1.1.1.40	C	Fair
Isocitrate dehydrogenase (*Idh*)	1.1.1.42	C	Good
6-phosphogluconate dehydrogenase (*6Pgdh*)	1.1.1.44	C,D	Fair
Glucose-6-phosphate dehydrogenase (*G6pdh*)	1.1.1.49	Poor	Good
Xanthine dehydrogenase (*Xdh*)	1.2.1.37	N.D.	Good
Superoxide dismutase (*Sod*)	1.15.1.1	C,D	Good
Asparate aminotransferase (*Aat*)	2.6.1.1	D	Fair
Glutamate pyruvate transaminase (*Gpt*)	2.6.1.2	C	Fair
Creatine kinase (*Ck*)	2.7.3.2	C	Good
Adenylate kinase (*Ak*)	2.7.3.2	C	Fair
Phosphoglucomutase (*Pgm*)	2.7.5.1	C	Good
Esterase (*Est*)	3.1.1.1	D	N.D.
Acid phosphatase (*Acp*)	3.1.3.2	C	N.D.
Fumarase (*Fum*)	4.2.1.2	N.D.	Good
Aconitase (*Aco*)	4.2.1.3	D	Fair
Mannose phosphate isomerase (*Mpi*)	5.3.1.8	C	Fair
Phosphoglucose isomerase (*Pgi*)	5.3.1.9	C	Fair
Muscle protein (*Prot*)	—	C	Good

monomorphic enzymes. Details of allelic frequency differentiation among populations appear in Ward et al. (1989). Three loci were unscorable in the zander samples; perhaps because these samples had been frozen for several months prior to analysis. A very slow aspartate aminotransferase and a fast phosphoglucomutase stained inconsistently and are not considered here.

Heterozygote banding patterns indicated that three of the enzymes were monomers: adenylate kinase, phosphoglucomutase, and esterase. Five were dimers: alcohol dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, phosphoglucose isomerase, and glucose-6-phosphate dehydrogenase. These quaternary structures correspond to those normally accepted for these enzymes (Hopkinson et al. 1976; Ward 1977). The single creatine kinase heterozygote was of the muscle-specific locus; the heterozygote was two banded, yet the enzyme is known to be dimer. This phenomenon is commonly observed in fish species, but its aetiology remains uncertain (Buth and Burr 1978; Ferris and Whitt 1978; Buth 1979). Muscle protein-4, encoded by *Prot*-4, is a monomer, and corresponds to the muscle myogen polymorphism described by Uthe and Ryder (1970), with alleles 100 and 160 corresponding to Uthe and Ryther's A and B, respectively. The previously undescribed polymorphic *Prot*-2 locus in walleye encodes a dimeric protein. The polymorphic fast migrating malate dehydrogenase isozymes in walleye (*Mdh*-3) correspond to the *Mdh*-C locus isozymes of Clayton et al. (1971, 1974), with alleles 70, 100, and 120 corresponding to Clayton's c1, c2, and c3, respectively.

Estimated mean heterozygosity per locus for walleye, between 5–6%, is three to four times those of the sauger and zander (Table 3).

Four of the 39 loci scored in walleye and sauger were species-diagnostic. These are sorbitol dehydrogenase (*Sdh*), mitochondrial malate dehydrogenase (*Mdh*-1), phosphoglucomutase-1 (*Pgm*-1), and glutamate pyruvate transaminase (*Gpt*). The first three of these loci have also been identified as species-diagnostic by Clayton et al. (1973), J. Clayton (unpubl. data) and T. Todd (unpubl. data); the fourth locus was not screened by these workers. Many more loci are diagnostic between the two North American species and the European zander. Fifteen of the 36 loci scored in walleye and zander were species-diagnostic, as were 14 of 36 loci scored in sauger and zander.

Nei's unbiased genetic distance (Nei 1978) and Roger's genetic distance (Rogers 1972) for the three possible pairwise comparisons for the 36 loci scored in each of the species were determined (Table 4). The standard errors of Nei's distances are calculated as $[(1-I)/In]^{0.5}$ where I is the genetic identity of the pair of species and n the number of loci screened. Note that genetic distances are much more severely affected by the number of loci sampled than by the number of individuals surveyed (Sarich 1977; Nei 1978; Gorman and Renzi 1979). A UPGMA phenogram constructed from the Nei distances (Fig. 1a) and an unrooted Wagner tree generated from Roger's distances (Fig. 1b) both showed that walleye and sauger were more closely related to each other than either was to the zander. Nei's unbiased genetic distance over all 39 loci scored in walleye and sauger is 0.143 (SE = 0.068), and thus similar to the figure estimated from the 33 common loci (0.164 ± 0.070).

Mitochondrial DNA

Restriction fragment patterns have already been detailed for walleye mtDNA (Billington and Hebert 1988) and for sauger mtDNA (Billington et al. 1988), but there is no prior work on zander. Nineteen of the 24 endonucleases employed in the present study cut zander mtDNA at least once; sites were absent for

TABLE 2. Tissues screened and allele frequencies at each locus. Four sauger and two zander were screened. The numbers of walleye screened are given in parentheses for each locus.

Locus	Tissue	Allele	Allele frequencies: Walleye	Sauger	Zander
Adh	Liver	−60	0.136 (162)	—	—
		−100	0.864	1.0	1.0
αGpdh-2	Liver	100	1.0 (36)	1.0	1.0
αGpdh-1	Muscle	100	1.0 (60)	1.0	1.0
Sdh	Liver	−10	— (76)	1.0	—
		100	1.0	—	—
		120	—	—	1.0
Ldh-3	Eye	100	1.0 (38)	1.0	—
		105	—	—	1.0
Ldh-2	Any	65	— (63)	—	1.0
		100	1.0	1.0	—
Ldh-1	Any	75	— (63)	—	1.0
		100	1.0	1.0	—
Mdh-3	Muscle	70	0.009 (162)	—	—
		100	0.673	—	—
		120	0.318	1.0	1.0
Mdh-2	Any	100	1.0 (162)	1.0	1.0
Mdh-1	Muscle	100	1.0 (162)	—	—
		140	—	1.0	—
		200	—	—	1.0
Me	Muscle	90	— (35)	—	1.0
		100	1.0	1.0	—
Idh-2	Muscle	100	1.0 (46)	1.0	1.0
Idh-1	Liver/eye	75	0.306 (162)	—	—
		100	0.694	1.0	—
		120	—	—	1.0
6Pgdh	Any	100	1.0 (17)	1.0	1.0
G6pdh	Eye	100	1.0 (26)	1.0	0.75
		125	—	—	0.25
Xdh	Liver	100	1.0 (41)	1.0	–
		115	—	—	1.0
Sod	Liver	100	1.0 (75)	1.0	—
		160	—	—	1.0
Aat-2	Muscle	85	— (40)	—	1.0
		100	1.0	1.0	—
Aat-1	Liver	100	1.0 (36)	1.0	?
Gpt	Liver	85	— (17)	1.0	1.0
		100	1.0	—	—
Ck-3	Eye	100	1.0 (30)	1.0	1.0
Ck-2	Muscle	80	0.003 (157)	—	—
		100	0.997	1.0	1.0
Ck-1	Liver/eye	100	1.0 (26)	1.0	1.0
Ak	Muscle	100	0.996 (119)	1.0	1.0
		140	0.004	—	—
Pgm-1	Muscle/liver	80	0.004 (138)	1.0	—
		100	0.996	—	1.0
Est-3	Liver	100	1.0 (29)	1.0	?
Est-2	Muscle/liver	100	1.0 (35)	1.0	1.0
Est-1	Liver	100	1.0 (34)	0.50	—
		105	—	0.50	1.0
Acp-2	Liver	75	— (39)	—	1.0
		100	1.0	1.0	—
Acp-1	Liver	100	1.0 (39)	1.0	1.0
Fum	Muscle	100	1.0 (32)	1.0	1.0
Aco	Liver	100	1.0 (31)	1.0	?
Mpi	Muscle/liver	100	1.0 (20)	1.0	1.0
Pgi-2	Muscle/liver	85	— (146)	—	1.0
		90	0.003	—	—
		100	0.997	1.0	—
Pgi-1	Muscle	100	1.0 (146)	1.0	—
		140	—	—	1.0
Prot-4	Muscle	100	0.624 (161)	—	0.50
		160	0.376	1.0	0.50
Prot-3	Muscle	100	1.0 (75)	1.0	1.0
Prot-2	Muscle	60	0.307 (161)	—	—
		100	0.693	1.0	1.0
Prot-1	Muscle	−100	1.0 (81)	1.0	1.0

TABLE 3. Average heterozygosities per locus for walleye, sauger, and zander. *N* = mean number of individuals typed per locus. Ho = observed mean heterozygosity per locus. He(1) = expected mean heterozygosity per locus. He(2) = unbiased (Nei 1978) expected mean heterozygosity per locus.

	36 Common loci				39 Loci			
Species	*N*	Ho	He(1)	He(2)	*N*	Ho	He(1)	He(2)
Walleye	80.5	0.053	0.057	0.057	76.7	0.049	0.052	0.052
Sauger	4.0	0.009	0.014	0.016	3.9	0.009	0.013	0.014
Zander	2.0	0.034	0.024	0.032	—	—	—	—

TABLE 4. Genetic distances among walleye, sauger, and zander based upon the 36 loci scored in common. Nei's (1978) unbiased genetic distances (with SE) are given above the diagonal, with Rogers' (1972) genetic distances below.

	Walleye	Sauger	Zander
Walleye	—	0.164 (0.070)	0.588 (0.149)
Sauger	0.182	—	0.527 (0.139)
Zander	0.455	0.424	—

Bst EII, *Cla* I, *Mlu* I, *Pvu* I, and *Pvu* II. Thirteen of these 19 endonucleases cut zander mtDNA more than once (Table 5), while the remainder (*Bgl* II, *Eco* RI, *Kpn* I, *Sal* I, *Sma* I, *Sst* I) each produced a single cut. No polymorphisms were found among the three zander with any of the endonucleases. The length estimate for the zander mtDNA molecule averaged 16 790 ± 60 base pairs (bp) (Table 5), a value similar to that of walleye, sauger, and other fish species (Billington and Hebert 1988). Forty-eight restriction sites (representing 1.72% of the genome) were resolved on the zander mtDNA molecule with the 24 endonucleases (Table 6), while 57 sites were detected in both walleye and sauger representing approximately 2% of their genomes.

Initially, the number of restriction fragments produced by each endonuclease in the three species was determined (Table 7). The endonuclease *Pvu* I failed to cut the mtDNA of

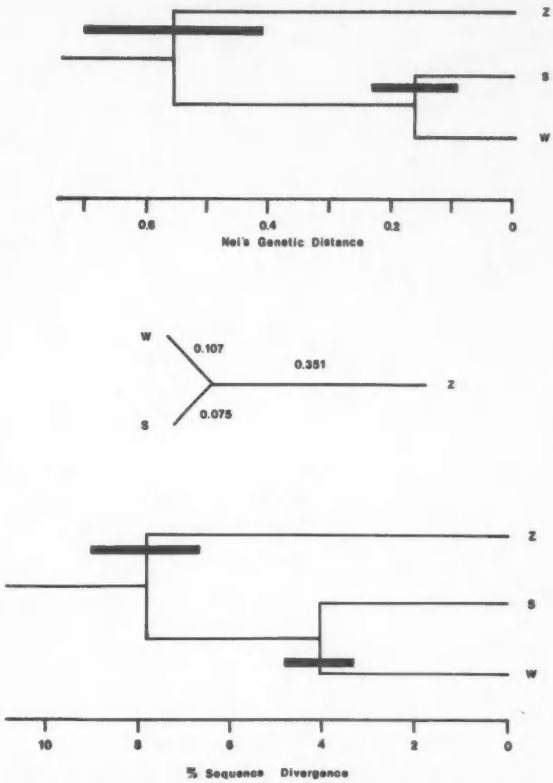

FIG. 1. Phylogenetic trees of relationships between sauger, walleye, and zander generated from allozyme data (a,b) and mtDNA restriction site data (c): (a) Nei unbiased genetic distance UPGMA phenogram with branch points ± 1 SE; (b) Roger's genetic distance unrooted Wagner network, with branch lengths; (c) UPGMA phenogram of percent sequence divergence (plotted as D/2), with branch points ± 1 SE.

TABLE 5. Fragment sizes (kilobase pairs) of zander mtDNA for those restriction endonucleases cutting more than once. Total length of molecule, as determined for each enzyme, are also given.

Enzyme	Fragment size	Total length
Apa I	10.11, 4.10, 2.6	16.81
Ava I	5.28, 3.20, 2.95, 2.19, 1.77, 0.98, 0.38	16.75
Bam HI	16.62, 0.14	16.76
Bcl I	7.97, 6.18, 2.61	16.76
Dra I	8.65, 5.26, 2.88	16.79
Eco RV	13.35, 3.45	16.80
Hind III	10.81, 2.35, 1.78, 1.72	16.66
Nco I	8.00, 3.30, 3.10, 2.36	16.76
Pst I	8.02, 6.98, 1.82	16.82
Sca I	15.56, 1.22	16.78
Sst II	9.17, 5.58, 1.75, 0.26	16.76
Xba I	4.85, 4.75, 2.51, 2.16, 2.02, 0.66	16.95
Xho I	14.68, 2.13	16.81
Mean length = (± *s*) (*n* = 13)		16.79 ± 0.06

TABLE 6. Number of restriction sites produced by hexanucleotide sequence recognition endonucleases in walleye, sauger, and zander mtDNA, total number of nucleotides examined and percentage of the genome surveyed. Note: Values in the percent of genome surveyed column are based on estimated genome sizes of 16 790 bp. for sauger (Billington et al. 1988), 16 860 bp. for walleye (Billington and Hebert 1988), and 16 785 bp. for zander (Table 5).

Species	Total number of cut sites	Total number of nucleotides examined	Percent of genome surveyed
Sauger	57	342	2.04
Walleye	57	342	2.03
Zander	48	288	1.72

TABLE 7. Number of restriction sites and number of common restriction sites revealed by digestion of walleye, sauger, and zander mtDNA with 24, six-base restriction endonucleases.

Enzyme	Walleye	Sauger	Zander	W/S	W/Z	S/Z
Apa I	3	3	3	1	1	1
Ava I	13	8	7	8	3	3
(*Sma* I	3	1	1	1	1	1[a]
(*Xho* I	2	2	2	—	—	—[a]
Bam HI	2	3	2	1	1	1
Bcl I	4	3	3	3	2	2
Bgl II	—	1	1	—	—	—
Bst EII	1	1	—	—	—	—
Cla I	—	—	—	—	—	—
Dra I	3	3	3	2	—	—
Eco RI	1	1	1	—	—	—
Eco RV	4	5	2	4	1	1
Hind III	5	6	4	5	4	4
Kpn I	1	1	1	—	—	1
Mlu I	1	2	—	1	—	—
Nco I	4	2	4	1	1	1
Pst I	3	2	3	2	1	—
Pvu I	—	—	—	—	—	—
Pvu II	1	—	—	—	—	—
Sal I	2	1	1	1	1	1
Sca I	4	4	2	2	—	—
Sst I	—	1	1	—	—	1
Sst II	3	4	4	3	3	3
Xba I	2	6	6	1	1	3

[a]*Sma* I and *Xho* I each recognize one of the four *Ava* I recognition sites, hence results for these enzymes are presented below *Ava* I.

any of the species, while a single *Cla* I site was found in two walleye (of 141 surveyed by Billington and Hebert 1988), but not in sauger or zander. Restriction site maps were constructed (Fig. 2) in order to compare the number of shared and unique restriction sites found among the three *Stizostedion* species (Tables 7 and 8); sites falling within 0.5 map units (approximately 80 bp) of each other were considered homologous. Eighteen restriction sites were common to all three species. In addition, walleye and sauger shared a further 17 sites, walleye and zander a further two sites, and sauger and zander a further five sites. The walleye restriction map shown is that of clone 1 (Billington and Hebert 1988), although the map origin and the orientation have been changed from that earlier publication. In addition, a *Sca* I site, erroneously included in the previous walleye map at position 64 has been deleted.

MtDNA percent sequence divergence (p) (Nei et al. 1985) was calculated among the three *Stizostedion* species (Table 8) and a UPGMA phenogram generated from these data (Fig. 1c). A Wagner network calculated using PAUP (Swofford 1985) had

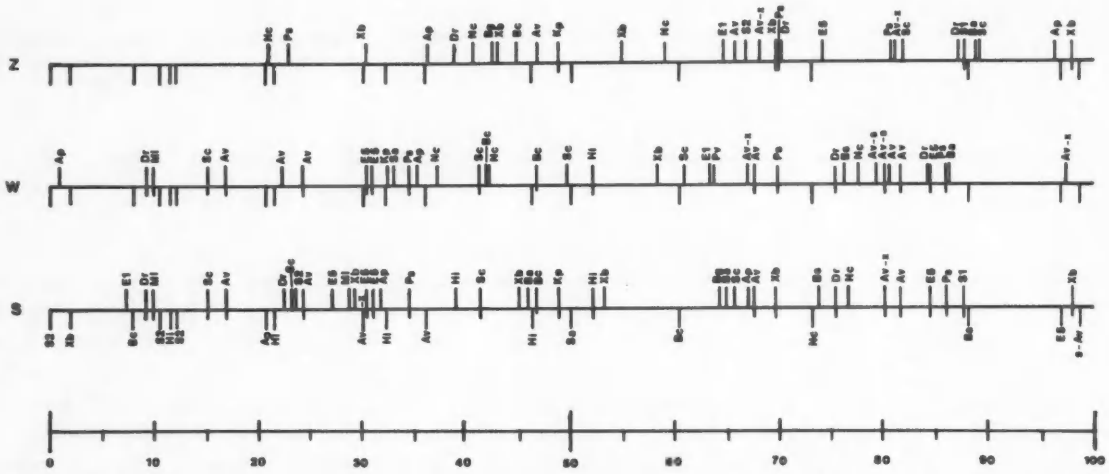

FIG. 2. Restriction site maps of sauger, walleye, and zander mtDNA molecules. Ap = *Apa* I, Av = *Ava* I, Ba = *Bam* HI, Bc = *Bcl* I, Bg = *Bgl* II, Bs = *Bst* EII, Dr = *Dra* I, El = *Eco* RI, E5 = *Eco* RV, Hi = *Hind* III, Kp = *Kpn* I, M1 = *Mlu* I, Nc = *Nco* I, Ps, *Pst* I, Pv = *Pvu* II, Sa = *Sal* I, Sc = *Sca* I, S1 = *Sst* I, S2 = *Sst* II, Xb = *Xba* I. For *Ava* I, Av-s, and Av-x designate sites also recognized by *Sma* I and *Xho* I, respectively. A map scale of 0–100 arbitary units is included. Sites common to all three species are shown below the map lines, those common to two of the species are shown to cross the lines and unique sites are shown totally above the lines.

TABLE 8. Matrix of mtDNA percent sequence divergence, *p* (with SE), (above diagonal) and numbers of shared restriction sites used to generate these distances (below diagonal) for walleye, sauger, and zander mtDNA.

Species	Walleye	Sauger	Zander
Walleye	57	8.13 (1.46)	16.94 (2.76)
Sauger	35	57	14.50 (2.41)
Zander	19	22	48

identical topology to the UPGMA phenogram. Moreover, inclusion of intraspecific comparison data for walleye (Billington and Hebert 1988) in both the phenetic and cladistic analyses had no significant effect on the calculated distances, and no effect whatsoever on the branching order obtained.

Discussion

Allozyme Data

The mean heterozygosity per locus of the walleye, at between 5–6%, is similar to the average value of 5.1% claculated by Nevo et al. (1984) from 183 fish species. The sauger and zander appear to be rather less variable, with average heterozygosities of approximately 2%. Walleye population sizes are substantially greater than those of sauger in the Great Lakes region, and its range is also greater (Scott and Crossman 1973). Zander used in this survey were taken from an English population which was established by introductions from Germany about 100 years ago (Rickards and Fickling 1979); it remains an infrequent species in England today. Thus, it seems very likely that the effective population size of the walleye is substantially greater than that of either the sauger or the zander, and this may be reflected in their greater heterozygosity. Although the sample sizes of zander and sauger were considerably less than those of walleye, average heterozygosity estimates are far more dependent on the number of loci sampled than on sample size. Reasonable numbers of loci were sampled in all species, so the reduced heterozygosities of sauger and zander are unlikely to be an artifact of small sample size.

It is clear from consideration of the numbers of species-diagnostic loci, from the genetic distance matrix (Table 4) and from the trees constructed (Figs. 1a,b), that the walleye and sauger are more closely related to one another than either is to the zander. Of course, it is perhaps not surprising that the North American species should be more closely related to each other than to the European species. Of more interest is the use that can be made of these genetic distance estimates in arriving at divergence times.

The most frequently used calibration of the allozymically-derived molecular clock indicates that one unit of Nei's genetic distance (*D*) is approximately equivalent to 19 million years (Carlson et al. 1978; Vawter et al. 1980). Empirical support for such a calibration in determining fish divergence times has been provided by Vawter et al. (1980), and has been used in a number of other fish studies (e.g. Galleguillos and Ward 1982; Grant et al. 1984). Its use on the present data indicates that the walleye and sauger lineages separated 3.12 (±1.33 SE) million years before present (MYBP), while the North American taxa and the zander separated 10.59 (±2.74) MYBP. These figures are derived from the distances calculated from the 36 loci scored in all species. If the three extra loci scored between walleye and sauger are taken into account, the estimated divergence for these two species changes slightly, to 2.85 (±1.22) MYBP. These divergence times can only be regarded as 'ball-park' estimates, partly through assumptions of the molecular clock itself (Korey 1981), partly through possible errors in calibrating the clock and partly because different enzymes have different evolutionary rates (Sarich 1977; Skibinski and Ward 1982). The clock should be calibrated anew for each user depending on the particular mix of enzymes used, but no suitable correction factors have yet been published.

Mitochondrial DNA

The restriction site data indicate that sauger and walleye are more similar to each other than is either species to the zander

(Fig. 2, Table 7). The values for percent sequence divergence of mtDNA (p) between the walleye and sauger (8.13%), between sauger and zander (14.5%),and between walleye and zander (16.9%) are compatible with other comparisons of intrageneric divergence. For example, Tegelström et al. (1988) list interspecific mtDNA sequence divergence values ranging from 1–16%, while Ferris and Berg (1987) report values ranging from 2.4–20.6%. Values for interspecific comparisons of fish within the same genus range from 2.5–14.0% for salmonids (Berg and Ferris 1984; Thomas et al. 1986; Gyllensten and Wilson 1986; Grewe et al. 1990), while the median sequence divergence between *Lepomis* species was 20.6% (Avise and Saunders 1984). In other studies of interspecific mtDNA variation in fish, Ovenden et al. (1988) calculated the net sequence divergence as 6.6% between *Gadiopsis marmoratus* and *G. bispinosus*, Becker et al. (1988) noted 11.6% divergence between *Merluccius capensis* and *M. paradoxus*, and Avise et al. (1987) recorded 10% sequence divergence between the toadfish *Opsanus tau* and *O. beta*.

Ferris et al. (1981) recommend the use of restriction enzymes that recognize more than one site in restriction site mapping, especially when they are used in conjunction with "semi-isoschizomers" (enzymes recognizing subsets of the possible sequences recognized by a multiple recognition sequence endonuclease). In the present study, the endonuclease *Ava* I was used which recognizes four sequences (CCCGAG, CTCGGG, CCCGGG, CTCGAG). *Sma* I recognizes one of those sequences (CCCGGG) and *Xho* I another (CTCGAG). The latter two endonucleases were particularly useful in mapping the large number of *Ava* I restriction sites found in *Stizostedion* mtDNA (seven in zander, eight in sauger, and 13 in walleye). However, the restriction site maps (Fig. 2), show that when *Ava* I sites are shared between the species, the sub-set composition of the *Ava* I site sometimes varies. For instance, at map position 30, both walleye and sauger share an *Ava* I site (confirmed by running digests and double digests of the two species in adjacent lanes of the gel), yet the sauger site alone is also recognized by *Xho* I. Thus, there is a minimum of one base change within the sauger *Ava* I site as compared with (presumably) the same site in walleye. Similarly, at map position 81 there is another shared *Ava* I site between walleye and sauger, but in this case the sauger site is also recognized by *Xho* I, whereas the walleye site is recognized by *Sma* I. The recognition sequences of these latter two endonucleases differ by two nucleotide substitutions. It is, of course, impossible to tell if other shared *Ava* I sites have differences of one to two mutations among them, as semi-isoschizomers are not available for the other two *Ava* I recognition sequences.

Taking into account the nucleotide changes in the above example, we have underestimated the sequence divergence between walleye and sauger by at least three site changes. Even so, we decided to calculate percent sequence divergence based on *Ava* I sites alone as we are unable to detect the true sequences of the *Ava* I sites for which no semi-isoschizomers exist. Thus, our current percent divergence values should be considered as conservative. However, the preceeding discussion makes it clear that measures of sequence divergence made using multiple sequence recognition restriction endonucleases are likely to be underestimates, because changes at hypervariable sites may go undetected.

The p values obtained between sauger and zander (14.5%), and between walleye and zander (16.9%) are at the upper limit of the range over which restriction site maps are useful for phylogenetic analyses. Once p values exceed 15%, resolution is reduced by homoplasy, as the rapidly evolving sequences become saturated with substitutions and multiple events may have occurred at many sites (Moritz et al. 1987). Bearing in mind this caveat, and noting the limitations of the universal applicability of "mtDNA clocks" (Moritz et al. 1987), we estimated divergence times between the walleye, sauger, and zander by assuming that the molecular clock calibration of Brown et al. (1979) of approximately 2% divergence (1% per lineage) per million years can be applied to *Stizostedion* mtDNA. Other studies using a number of vertebrate species generally corroborate this value (Wilson et al. 1985, and references therein; Shields and Wilson 1987), which has been used to estimate divergence times among salmonids (Thomas et al. 1986; Grewe et al. 1990), walleye mtDNA clones (Billington and Hebert 1988), and other fish species (Bermingham and Avise 1986). Thus, the walleye and sauger lineages diverged approximately 4.07 (±0.73 SE) MYBP, with the North American taxa separating from the European zander approximately 7.86 (±1.15) MYBP.

TABLE 9. Approximate divergence times (million years before present ± SE) for North American and European *Stizostedion* species based on allozyme and mtDNA data.

	Allozyme	mtDNA
Walleye and Sauger	3.12±1.33 (36 loci) 2.85±1.22 (39 loci)	4.06±0.73
Walleye/Sauger and Zander	10.59±2.74 (36 loci)	7.86±1.18

Comparison of Allozyme and Mitochondrial DNA Data

Similar conclusions concerning the evolution of walleye, sauger, and zander are obtained with allozyme and mtDNA data, estimates of divergence times obtained by the two methods are reasonably concordant (Table 9). In fact, given that the standard errors associated with each dichotomy do not overlap, the divergence times given by the two methods are not significantly different. Indeed, the apparent (though non-significant) reduction of divergence times of the North American species from the European species may be caused by an underestimate of the mtDNA divergence value, as the d value used was at the limit of the linear portion of the clock curve, and thus subject to homoplasy (see above). Furthermore, limited allozyme and mtDNA data on *Stizostedion volgense*, also from Europe, suggest that this species is more closely related to the European zander than it is to the two North American species (N. Billington and R. D. Ward, unpubl. data).

Other protein clocks fit less well with the mtDNA clock. For example, protein clocks in which one unit of D is equivalent to 5 million years divergence (Nei 1975) would yield a divergence time of 2.78 (±0.72) MYBP for the European/North American split, substantially less than the mtDNA estimate of 7.86 (±1.15) MYBP.

Similar studies on other fish species are scarce. Using the same proportionality constants that we used, Grant et al. (1988) and Becker et al. (1988) compared protein and mtDNA divergence times for two species of southern African hake, *Merluccius capensis* and *M. paradoxus*. They obtained estimates of 11.1 (±3.0) MYBP and 5.8 (±1.8) MYBP, respectively. These estimates do not correspond to one another as closely as our estimates, and Becker et al. (1988) suggested that the dif-

ferences between their two estimates may indicate that the calibrations of the mtDNA clock based essentially on primate data may be inappropriate for lower vertebrates, or that it reflects errors inherent in each method. Of course, it may also be that the protein clock is not accurately calibrated. More work comparing allozyme and mtDNA clocks is needed, although it is worth pointing out that Tegelström et al. (1988) have already demonstrated a highly significant correlation (Spearman rank correlation coefficient of 0.84, $p<0.001$) between mtDNA divergence and protein distance in 26 species pairs (25 mammal pairs, mostly *Mus*; and one pair of amphibians). Kessler and Avise (1985) also examined the relationship of p and D in a number of taxa, including the fish genus *Lepomis*. They found that small mtDNA distances observed among bird genera corroborated previously documented small differences in avian allozyme distances. Kessler and Avise (1985) suggested that avian species might have shared a common ancestor more recently than their nonavian taxonomic counterparts, or that protein and mtDNA evolution must be somewhat decelerated in birds. However, Shields and Wilson (1987) calibrated mtDNA evolution in geese against fossil records and found that the approximate rate of 2% per million years ((Brown et al. 1979) applied to this avian group. Obviously, much more work is required in the area of calibrating molecular (e.g. allozyme, mtDNA) and paleontological estimates of evolution.

Colonization of North America by *Stizostedion*

Three contrasting hypotheses for the colonization of North America by *Stizostedion* have been advanced: an early (Miocene or before) Atlantic colonization (Lindberg, in Svetovidov and Dorofeeva 1963; Balon et al. 1977); a Pliocene colonization via Beringia (Collette and Banarescu 1977); and a late-Pleistocene Atlantic colonization (Cihar 1975). Despite the approximate nature of the divergence times obtained, it is very clear that only one of the three possible colonization hypotheses is compatible with the current molecular data. Presumptive Miocene or pre-Miocene colonization dates are not consistent with the 7–10 MYBP divergence values obtained between the European zander and the North American species. Furthermore, Lindberg's hypothesis was also rejected by Svetovidov and Dorofeeva (1963), because of the lack of geological evidence for a North Atlantic riverine connection.

We can reject Cihar's hypothesis concerning *Stizostedion* for three reasons. Firstly, the large genetic distances between the North American and European *Stizostedion* species, along with our divergence estimates of approximately 7–10 million years, are not compatible with divergence within the last 300 000–500 000 years. Secondly, our data suggest that the two North American species diverged 3–4 MYBP, long before the 300 000 years BP late-Pleistocene colonization time suggested by Cihar (1975). Thirdly, divergence estimates among groups of walleye mtDNA clones date to approximately 250 000 years BP (Billington and Hebert 1988), suggesting that walleye were already well established in North America by the late-Pleistocene.

Thus, our data are consistent with the hypothesis that *Stizostedion* colonized North America via Beringia during the Pliocene. The current data suggest that this colonization occurred approximately 5–10 MYBP, prior to the Bering land bridge becoming inundated 3–5 MYBP (Herman and Hopkins 1980). Furthermore, as walleye and sauger diverged approximately 3–4 MYBP, *Stizostedion* must have been well established in North America by the mid-Pliocene.

Acknowledgments

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Woody Debris, Channel Features, and Macroinvertebrates of Streams with Logged and Undisturbed Riparian Timber in Northeastern Oregon, U.S.A.

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Macroinvertebrate communities and several aspects of fish habitat were examined for 16 northeastern Oregon stream segments, 11 with undisturbed riparian forests and five where 26–54% of the riparian forest had been harvested 6 to 17 yr previously. Amounts of woody debris in streams and pools formed by the debris were similar between undisturbed and logged sites. Pool volume was inversely related to stream gradient and directly related to the amount of woody debris in the stream. Stream surface substrate composition was not significantly different between streams in logged and undisturbed areas. Macroinvertebrate density was 20 to 113 percent greater at the logged sites and diversity was similar at logged and undisturbed sites. Macroinvertebrates were most abundant at lower elevation streams and at streams that were shaded less by the surrounding vegetation. Timber harvesting activities do not appear to have damaged aquatic insect habitat and pool abundance was not altered, suggesting the habitat's carrying capacity for fish was not affected.

Les communautés de macro-invertébrés et divers aspects de l'habitat aquatique de 16 segments de cours d'eau du nord-est de l'Orégon, dont 11 en forêt riparienne non perturbée et 5 en forêt riparienne ayant subie une coupe de l'ordre de 26 à 54 pour cent de 6 à 17 années auparavant, ont été examinés. Les quantités de débris ligneux dans les cours d'eau et les fosses formées par les débris des sites non perturbés ou exploités étaient semblables. Le volume des fosses présentait une relation inverse avec le gradient du cours d'eau et une relation directe avec la quantité de débris ligneux se trouvant dans le cours d'eau. La composition du substrat de surface des cours d'eau ne différait pas de façon significative entre les deux types de sites. La densité de macro-invertébrés était de 20 à 113 pour cent supérieure aux endroits ayant subi une coupe, mais la diversité était semblable dans les deux types de sites. Les macro-invertébrés étaient les plus abondants dans les cours d'eau de faible élévation et dans ceux où la végétation avoisinante faisait moins d'ombre. Les coupes de bois ne semblent pas avoir nui à l'habitat des insectes aquatiques ni modifié l'abondance des fosses, ce qui porte à croire que la capacité de l'habitat à entretenir la vie du poisson n'a pas été modifiée.

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Small mountain streams in northeastern Oregon and southeastern Washington provide important spawning and rearing habitat for steelhead (*Oncorhynchus mykiss*) and chinook salmon (*O. tshawytscha*) that migrate up the Columbia and Snake Rivers. Dams on the Snake River have blocked fish passage to a great number of streams that were once available for spawning and so downstream tributaries are now even more critical for maintaining runs of wild fish.

Increased levels of timber harvesting within these drainages have led to concern that fish habitat is being damaged. Studies elsewhere (Bisson et al. 1987) have shown that harvesting streamside trees can deprive a stream of the woody debris needed to maintain pools and other channel features. Removing trees can also expose the stream to more sunlight, which can increase water temperature (Beschta et al. 1987). Roads and skid trails can, at times, be sources of fine sediments in the stream (Everest et al. 1987).

The severity of these changes and their influence on fish habitat were unknown for northeastern Oregon streams. In this study, we compared channel features and macroinvertebrate populations in stream segments having undisturbed riparian timber with those in segments having partially logged riparian stands. We measured the amount of woody debris in stream channels, the abundance of pools and other channel features, the degree of stream shading, and the abundance and variety of macroinvertebrates. Although we did not measure fish abundance directly, our evaluation of fish habitat can be used as an indicator of a stream's carrying capacity for fish (Platt et al. 1983).

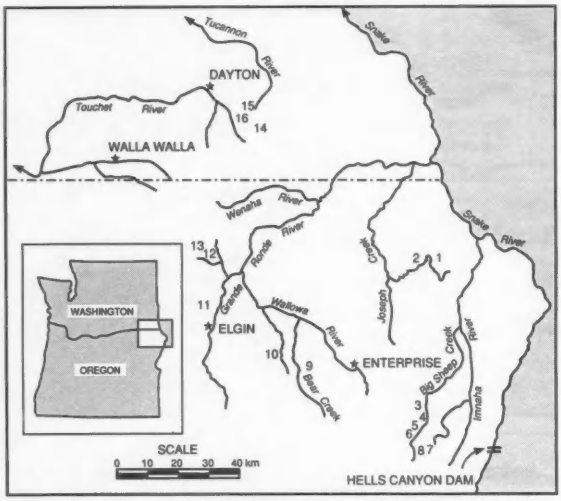

FIG. 1. Study area. Approximate location of sites indicated by numbers. Individual study streams not shown due to their small size.

Methods

Stream segments were selected from an area bounded to the east by the Snake River, to the south by the Eagle Cap Wilderness, and to the west by the town of Elgin, Oregon (Fig. 1). The study area extended north into southeast Washington and is within the intermountain region of the United States. The area is underlain by basalt, and streams form deep canyons with steep sides. Ridges are commonly broad and flat. The elevation of basins where timber harvesting occurs ranges from 900 to 1600 m.

The climate is a combination of maritime and continental. Mean annual precipitation is 50 to 80 cm with much of it falling as snow. Stream levels peak in May or June during snow melt. Precipitation and winter snow pack development varies greatly with elevation. Dry spells in the summer months are interrupted by brief rainfall during thunderstorms. January mean minimum temperature is −8°C and July mean maximum is 27°C.

Most forested land in this area is managed by the United States Forest Service, although some land at the lower elevations is privately owned. In our search for study sites we found that few trees had been harvested from the riparian areas of most fish-bearing streams. Most streams either had undisturbed riparian stands or had stands in which only a few of the more valuable trees had been removed. Past logging has rarely included removal of riparian timber, in part because the more valuable ponderosa pine and larch commonly grow on slopes away from the riparian zone. Today, more of the less-valuable species such as Engelmann spruce and grand fir commonly found in riparian zones are being harvested. However, wide buffers of uncut timber are usually left next to fish-bearing streams on Forest Service land.

We eventually selected five stream segments that had one-quarter to one-half of their riparian stands (the area within 20 m of stream centerline) removed. Table 1 describes the dates and types of logging for these sites. These five streams represent a level of riparian stand removal that is probably higher than will occur here on Forest Service land in the future, except perhaps during the salvage logging of burned or insect-killed stands.

TABLE 1. Description of logging in logged sites.

Site	Description
1	High winds blew down most of stand on north side of stream in 1968. Cable logged in 1970. Mostly clearcut on north side; light partial cut on south side.
3	Tractor logged in 1981. Heavy partial cut both sides of stream. All merchantable timber cut. Remaining trees were mostly small grand fir.
5	Cable logged in 1978. For half of study segment most trees were harvested on both sides of stream. For other half only the southwest side of stream was cut. Scattered trees were left in the cut areas.
7	Cable logged in 1974. Heavy partial cut on east side of stream. Most large merchantable trees cut (larch, Douglas-fir, spruce). Remaining trees mostly smaller spruce. Most large snags near stream were felled across stream before logging.
15	Tussock moth outbreak killed many riparian trees prior to tractor logging in1971. Mostly clearcut on north side of stream with narrow buffer; light partial cut on south side.

We paired each logged stream segment with a nearby stream segment that had similar characteristics but had an undisturbed riparian stand. Paired segments were either on the same stream or were on adjacent streams that were similar. When paired segments were on the same stream the logged segment was downstream of the undisturbed segment. Six additional undisturbed stream segments, representing a range of stream and stand conditions, were also selected for study.

In this study the term undisturbed refers to the condition of the riparian stand. In nearly all watersheds there was some upstream disturbance from logging and cattle grazing. However, upstream logging rarely encroached upon the riparian area. Roads were often located on ridges and were not obvious sources of stream sediment. Skid trails, where they occurred, were waterbarred and showed little sign of erosion. Cattle grazing is common within riparian areas of this region. Grazing intensity was greatest along lower elevation streams or where tree removal had increased grass growth. Grasses and forbs were noticeably shorter at the sites where cattle had been grazing during the summer but we saw no evidence of widespread bank disturbance because of this grazing.

Drainage areas upstream of the selected stream segments varied from 7 to 25 km^2 and elevations at the stream segments ranged from 945 to 1550 m (Table 2). Stream segments were 300 m long, except at sites 1 and 2, where segments were 240 m. Eleven sites supported runs of steelhead and five sites (12, 13, 14, 15, 16) supported runs of chinook salmon. Field measurements were completed during the summer of 1987.

We made channel measurements at transects spaced 10 m apart. Measurements included summer stream width, bankfull stream width (defined by vegetation or an abrupt change in side slope), and surface substrate composition (visual estimate of the percentages of silt and sand, gravel, cobbles, and boulders).

Pools and riffles were measured and classified throughout each stream segment. Pools were defined as areas of relatively still water where the stream bottom was concave and both the length and width were at least half of the summer stream width at that point. All other channel features were defined as riffles. Pool depth was determined by averaging depth measurements taken at three or four locations near the edges and at one location in the center. We also recorded the features responsible for

TABLE 2. Watershed and channel characteristics.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Pairs[a]	a	a	b	b	c	c	d	d	—	—	—	—	—	—	e	e
Riparian stand[b]	L	U	L	U	L	U	L	U	U	U	U	U	U	U	L	U
Drainage area (km^2)	10	25	14	19	15	9	16	8	7	12	16	25	7	19	13	12
Elevation (m)	1400	1190	1220	1310	1490	1550	1520	1520	1550	1430	1070	1130	1310	1100	945	975
Gradient (%)	2.8	2.0	4.5	4.4	5.6	6.5	3.0	7.1	7.4	4.5	2.1	3.8	5.6	3.5	5.2	5.4
Summer riffle depth (cm)	6	5	9	6	6	7	5	8	9	14	4	9	5	6	6	10
Summer width (m)	1.7	2.2	2.3	3.1	2.2	2.6	2.7	2.4	2.3	2.9	2.3	3.0	2.1	3.3	2.2	1.9
Bankfull width (m)	2.1	4.1	3.0	4.5	3.6	3.8	5.8	3.8	3.0	4.1	4.0	6.1	4.0	4.5	2.6	2.3
Substrate (%):																
Silt and sand	27	13	12	10	14	18	16	11	5	7	20	12	23	20	13	21
Gravel	56	46	50	47	42	32	52	48	52	44	45	40	30	49	36	38
Cobbles	16	35	31	30	26	20	25	29	36	40	34	35	25	30	45	33
Boulders	1	6	7	13	18	30	7	12	7	9	1	13	22	1	6	8

[a]Sites with the same letter are paired.
[b]L = logged site, U = undisturbed site.

creating the pool, such as logs, boulders, bedrock or stream meanders.

We inventoried live trees, snags, and stumps larger than 15 cm in diameter at breast height (DBH) in a swath that extended 20 m from each side of stream centerline for the entire stream segment. Snags were defined as dead standing trees at least 3 m in height. The heights of a selected number of live trees were measured to determine the relationship between diameter and height for each tree species. Stump diameter was also measured on selected trees to determine the relationship between stump diameter and DBH. These relationships were used to reconstruct the stand at logged sites. Equations developed by Wykoff et al. (1982) were used to calculate tree volume using DBH and height.

An angular canopy densiometer was used to determine the shading provided by vegetation south of the stream centerline (Summers 1982). Separate measurements were made for shade provided by the overstory and by the understory, and a combined measurement was taken as well. A spherical canopy densiometer (Lemmon 1957) was used to measure overstory and understory canopy cover in all directions. Shade and canopy measurements were made in the center of the channel at 10-m intervals and averaged for each stream segment.

Woody debris was inventoried in a strip extending 10 m from each side of stream centerline. Only debris that was both greater than 10 cm in diameter and at least 1 m in length was included. Diameter and length were measured for some pieces and were estimated for others. Periodic measurements of estimated lengths or diameters helped to maintain accuracy. If a portion of a piece of wood lay outside the 10-m sampling swath, that portion was not included in the inventory. Each debris piece was classified in four ways: (1) by species, (2) by whether it was or was not part of a clump, (3) by the percentage of the piece's volume that was within the high water marks, and (4) by its effect, if any, on the stream.

In mid-June of 1987, macroinvertebrates were sampled at each stream segment within the riffles upstream of five randomly chosen transects. A "kick sampling" technique was used to collect the insects (Pollard 1981; Frost et al. 1971). A 35.5-cm D-frame net with a 0.8×1.2-mm mesh was positioned vertically with the lower rim resting on the stream bottom and the current flowing into the net. The substrate in front of the net was kicked for 2 min as the net was moved upstream about 2 m. The sample was preserved in an alcohol/formalin solution in the field and sorted in the lab. Most of the Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera were identified to genus or species. Several Diptera were identified only to the family level (Carlson 1989). The Shannon-Wiener diversity index (Wilhm 1970) was used to describe community diversity. It was computed for the lowest operational taxa, using consistent taxa classification across sites.

Throughout this study the phrase "significantly different" corresponds to differences that are significant with p less than 0.05. Similarly, variables are not included in regression equations unless their significance level (p) is less than 0.05. Coefficients of multiple determination (R^2) presented with equations are adjusted for the number of variables in the equation. Unless otherwise noted, mean values are compared with a paired t-test.

Results and Discussion

The Riparian Forest

Most of the trees growing within 20 m of stream centerline at the undisturbed sites and at the logged sites (before they were harvested) were Engelmann spruce (*Picea engelmannii*), Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), western larch (*Larix occidentalis*), and subalpine fir (*Abies lasiocarpa*). Their relative abundance varied among sites (Table 3). Stands were commonly two-tiered, with large trees scattered among many smaller trees. Stand density and species composition in these riparian stands often varied widely within the study reaches. Variations in soil rockiness, slope steepness, terrace width, mortality from disease, and beaver (*Castor canadensis*) activity contributed to this patchy distribution of trees. Conifers commonly did not grow to the edge of the stream channel. The width of the corridor of land next to the stream that was without conifers ranged from 5 to 34 m among sites. The mean width of this corridor was 15 m. Steamside stands rarely included hardwood trees larger than 10 cm DBH. Common riparian shrubs were alder (*Alnus sinuata* and *A. tenuifolia*) and western dogwood (*Cornus stolonifera*).

Stand basal area (before cutting on the logged sites and including snags at all sites) ranged from 20 to 66 $m^2 \cdot ha^{-1}$ and averaged 38 $m^2 \cdot ha^{-1}$. Snag basal area ranged from 12 to 29% of total basal area at the undisturbed sites and averaged 19%. These values were similar to those found in mature stands in the Oregon Coast Range, where snag basal area averaged 16%

TABLE 3. Riparian stand characteristics.

Site	Pairs[a]	Stand density (trees·ha;-s[1]) Live trees	Snags	Cut trees	Basal area ($m^2 \cdot ha^{-1}$) Live trees	Snags	Cut trees	Gross volume ($m^3 \cdot h^{-1}$) Live trees	Cut trees	Dominant species[b]	Corridor width (m)[c]
1	a	126	16	51	15	4	9	178	122	DF,SP,LA	17
2	a	146	40	0	17	7	0	193	0	DF,LA,SP	21
3	b	159	28	81	10	7	21	105	292	WF,DF,SP	11
4	b	213	45	0	30	7	0	376	0	WF,DF,LA	20
5	c	140	25	84	11	5	17	124	203	SP,LA,DF	9
6	c	245	27	0	25	6	0	291	0	SP,LA,DF	8
7	d	169	16	40	14	2	13	154	185	SP,DF,LA	15
8	d	286	25	0	36	5	0	433	0	SP,LA,DF	11
9		616	127	0	44	17	0	462	0	SP,WF,LA	5
10		300	30	0	31	6	0	391	0	WF,SP,LA	14
11		63	11	0	17	3	0	253	0	SP,WF	34
12		298	30	0	55	11	0	805	0	WF,SP,DF	13
13		203	36	0	37	11	0	477	0	SP,DF,SU	18
14		230	13	0	35	5	0	478	0	WF,SP,YE	19
15	e	132	71	67	14	14	10	186	141	WF,PP,DF	11
16	e	329	31	0	31	6	0	382	0	WF,SP,DF	11

[a]Sites with the same letter are paired.
[b]Based on basal area of live trees, cut trees, and snags. Listed in decreasing order. DF = Douglas-fir, SP = Engelmann spruce, LA = western larch, WF = white fir, SU = subalpine fir, YE = pacific yew, PP = ponderosa pine.
[c]The width of corridor along stream that does not support conifers.

TABLE 4. Stream shading and canopy cover provided by riparian vegetation.

Site	Pairs[a]	Overstory angular canopy density (%)	Understory angular canopy density (%)	Combined angular canopy density (%)	Combined spherical canopy density (%)
1(L)[b]	a	24	12	32	53
2	a	65	11	68	63
3(L)	b	56	36	73	75
4	b	44	26	58	81
5(L)	c	21	13	33	54
6	c	58	13	62	70
7(L)	d	54	19	64	67
8	d	59	7	63	80
9		69	9	79	88
10		53	11	58	78
11		27	26	47	64
12		70	12	76	85
13		47	32	67	86
14		69	43	83	87
15(L)	e	52	59	83	82
16	e	69	48	86	93

[a]Sites with the same letter are paired.
[b](L) indicates logged site.

of total, and in the northern Cascades of Oregon, where snags averaged 21% of total (C. W. Andrus, unpubl. data).

Some sites had a number of dead larch trees but had few live ones because of an insect infestation that began about 25 yr ago. Also, a tussock moth (*Orgyia pseudotsugata*) outbreak that occurred about 20 yr ago killed many of the grand fir trees at sites 15 and 16.

Harvesting removed 26 to 54% of the basal area of the original stands (including snags) for the five logged sites. The average harvest was 41% of the total basal area. Harvested gross volume ranged from 141 to 292 $m^3 \cdot ha^{-1}$ and averaged 188 $m^3 \cdot ha^{-1}$. Most of the trees removed from streamside stands were larch and Douglas-fir. The majority of residual trees were smaller spruce, grand fir, and subalpine fir.

Shade and Canopy Density

Angular canopy density, a measure of shading by vegetation to the south, was not significantly different between logged and undisturbed streams (Table 4). It's possible that there were dissimilar levels of shading between paired streams before cutting. Increased understory growth during the years after cutting may have compensated for the decrease in overstory canopy. Also, tree removal did not result in decreased stream shading if most cutting occured on the north side of the stream, as was the case at site 15, or if harvesting was restricted to large trees scattered among numerous small trees, as was the case at site 7.

Spherical canopy density, a measure of canopy cover from understory and overstory vegetation in all directions, averaged 66% for logged sites and 77% for the paired undisturbed sites. These means were significantly different. We found a positive correlation between spherical canopy density and live tree basal area for undisturbed stream segments. However, at logged sites spherical canopy density was not correlated with residual basal area, since spherical canopy density had been heavily influenced by understory growth since logging. The increased sunlight made available by harvesting promoted rapid growth of understory species such as western dogwood and alder at sites 3 and 15.

We completed the measurements for this study in 1987. At that time only a few Engelmann spruce trees were infested with bark beetles (*Endroctonus engelmanni*). Since then, bark beetles have killed most of the spruce in the south portion of the study area. Spruce is the principal tree in many of the higher elevation riparian stands. Death of a portion of the stand (either by fire or insect infestation) is a periodic event in these riparian stands, as is demonstrated by the two-tiered structure of the current stands.

TABLE 5. Amounts of woody debris and pool characteristics.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Paired sites[a]	a	a	b	b	c	c	d	d	—	—	—	—	—	—	e	e
Riparian stand[b]	L	U	L	U	L	U	L	U	U	U	U	U	U	U	L	U
Pieces of debris 10 m each side of stream centerline ($\#\cdot ha^{-1}$)	470	390	530	380	700	490	525	400	715	450	305	255	500	250	755	355
Debris volume 10 m each side of stream centerline ($m^3\cdot ha^{-1}$)	155	140	205	180	260	150	230	90	255	145	235	250	285	195	260	130
Debris volume within high water marks ($m^3\cdot ha^{-1}$)	167	227	267	304	306	258	355	232	550	337	478	474	638	258	523	413
Percentage of pieces within high water marks that create pools	35	43	52	54	72	29	68	39	28	27	71	46	80	73	62	44
Number of pools ($\#\cdot 100m^{-1}$)	20	8	15	17	20	10	13	13	13	11	18	8	29	36	32	10
Percent channel area in pools	28	28	16	29	32	20	28	18	12	12	39	27	37	21	24	16
Pool:riffle ratio (l:x)	2.6	2.6	5.1	2.4	2.2	3.9	2.5	4.4	7.3	7.7	1.6	2.7	1.7	3.7	3.1	5.2
Mean pool volume (m^3)	0.3	1.4	0.4	0.6	0.6	0.5	1.2	0.4	0.4	0.6	1.0	2.0	0.3	0.5	0.3	0.5
Median pool volume (m^3)	0.2	0.9	0.3	0.3	0.3	0.3	0.6	0.3	0.2	0.3	0.4	1.4	0.2	0.4	0.1	0.4
Pool volume ($m^3\cdot ha^{-1}$)	324	538	252	369	498	194	563	229	233	231	755	541	475	385	351	284

[a]Sites with the same letter are paired.
[b]L = logged site, U = undisturbed site.

Woody Debris and Channel Features

Debris volume within 10 m of either side of the stream centerline ranged from 90 to 280 $m^3\cdot ha^{-1}$ and averaged 187 $m^3\cdot ha^{-1}$ for undisturbed stream segments (Table 5). By way of comparison, these values were about twice the debris volume measured by Knight (Environmental Protection Agency, New York, NY, unpubl. data) in central Oregon streams, yet were less than values for streams measured elsewhere in the Pacific Northwest. Heimann (1988) reported debris volumes averaging 380 $m^3\cdot ha^{-1}$ for Oregon coastal streams bordered by mature timber and Grette (1985) reported an average of 700 $m^3\cdot ha^{-1}$ for Washington coastal streams bordered by old-growth timber. Froehlich (1973) found that debris volume averaged 660 $m^3\cdot ha^{-1}$ in streams of the Oregon Cascades that were bordered by old-growth timber.

Woody debris volume (10 m each side of stream centerline) was related to snag basal area and density at undisturbed sites as shown by the equation:

$$\text{Woody debris volume } (m^3\cdot ha^{-1}) = 386 + 24.2\,[\text{snag basal area } (m^2\cdot ha^{-1})] - 112\,[\text{natural logarithm of snag density} \times (\text{trees}\cdot ha^{-1})],$$

$$R^2 = 0.65$$

The equation indicates that riparian stands with greater snag basal area will also have more woody debris on the ground. Furthermore, stands with lower snag density for a given snag basal area (fewer trees of larger size) will have more downed wood. Snag density in this case probably acts as an indicator of stand age or vigor.

The debris volume within 10 m each side of stream centerline at logged sites ranged from 1.1 to 2.5 times the debris volume found at the paired undisturbed sites. These increases were primarily due to logging residue and to the blowdown of trees since logging.

The volume of woody debris within the high water marks averaged 38% of total debris volume at the paired undisturbed streams. Values at logged sites were lower and averaged 25%; the lower values were probably a result of logging crews removing debris that had fallen into the channel during logging and placing it outside of the high water marks. Nevertheless, the volume of debris within the summer stream channel was not significantly different between paired, logged, and undisturbed sites.

Trees were seldom felled into the streams. At four of five sites, only 17% on average, of felled trees were directed towards the stream. At site 7, however, 56% of the trees were felled toward the stream because of steep slopes and the large size of the harvested trees.

On the average, 50% of the debris pieces that were within the high water marks were affecting the channel by forming pools (Table 5). In addition, 1% of the pieces were creating falls, 17% were restricting stream width, and 4% were diverting flow into a stream bank. The remaining 28% of the pieces had no affect on the stream channel. These percentages did not differ significantly between undisturbed and paired logged sites.

Debris pieces within the high water marks were classified according to their proximity to other pieces. An average for all the sites indicated that 23% were single pieces, 49% were in a loose association and 28% were part of a jam. The percent of pieces within jams ranged from 0% at sites 1 and 14 to 48% at site 12. Values for undisturbed sites did not differ from values at logged sites. However, streams with wider channels and higher gradients had a greater proportion of pieces in jams, reflecting the greater ability of larger and steeper streams to redistribute debris at high flows (Keller and Swanson 1979).

Debris acting alone or in conjunction with boulders was associated with 64% of the pools found in the 16 stream segments. Boulders alone created 24% of the pools, while the remainder

were created by other features. Results were similar to what Andrus et al. (1988) found for an Oregon coastal stream, where 70% of the pools were associated with woody debris.

Neither the number of pools per 100 m of stream, the mean pool volume, nor the percent area in pools differed significantly between paired logged and undisturbed stream segments. No correlation was found between percent area in pools and woody debris or boulder abundance. However, when pool volume was examined, woody debris abundance did help explain variation between sites as shown by the equation:

$$\text{pool volume } (m^3 \cdot ha^{-1}) = 268 + 959\ [\text{stream gradient}^{-1}(\%)] + 0.432\ [\text{debris volume within high water marks } (m^3 \cdot ha^{-1})] - 410\ [\text{ratio of summer stream width to bankfull width}] \qquad R^2 = 0.74$$

The equation indicates pool volume is greater at lower stream gradients and when there is more woody debris. The equation also indicates that sites with the greatest difference between spring and summer flows have the highest proportion of area in pools. Streams with a high spring flow to summer flow ratio are more likely to have deep pools scoured into their channels. Andrus et al. (1988) found similar results for a coastal Oregon stream where differences in pool volumes throughout a watershed were determined by channel size and gradient as well as woody debris abundance.

When we analyzed the residuals, we found no differences between logged and undisturbed sites. The residuals indicated that site 5 was an outlier. Actual pool volume was about twice the estimated value for this site. The unusually high pool volume at site 5 could be partly explained by large boulders within the stream that had created pools.

The percentage of stream substrate consisting of silt and sand ranged from 5 to 27% among sites (Table 2). A regression equation that included watershed area, summer riffle depth, and stream gradient explained about half of the variability between sites. No significant differences existed between logged and undisturbed sites. Stream surface substrate composition was no different at the logged sites 6 to 17 yr after harvesting than it was at the undisturbed sites with similar gradient, riffle depth, and area.

Macroinvertebrates

Total macroinvertebrate numbers were greater at logged segments than at undisturbed segments for paired streams (Table 6). These increases ranged from 20 to 113% and averaged 57%. This is less than the 2.5-fold increase that Newbold et al. (1980) found in northern California streams, where all streamside timber had been removed and only 1 to 5 yr separated timber harvesting from macroinvertebrate sampling. Murphy et al. (1981) noted two-fold increases in macroinvertebrates between streams with logged and undisturbed riparian zones when sampling was done 5 to 10 yr after timber harvesting. They attributed differences to increases in the growth of algae and vascular plants because of increased exposure to sunlight. The increases in macroinvertebrates in our study may be less than in other studies because harvesting removed only a portion of the riparian timber. In addition, the streams have become shaded by understory species that have flourished during the 6 to 17 yr since timber harvesting.

Samples were dominated by mayflies (*Ephemeroptera*), with smaller numbers of midges (*Diptera:Chironomidae*), caddisflies (*Trichoptera*), beetles (*Coleoptera*), stoneflies (*Plecoptera*), and true flies (*Diptera* excluding *Chironomidae*). Generally, the taxa present in undisturbed streams were also present in logged streams. Likewise, no differences in the number of taxa between clearcut and forested riparian zones were found by Newbold et al. (1980) in northern California and Noel et al. (1986) in northern New England.

No significant differences in diversity, as expressed by the Shannon-Weiner Index, were found between logged and paired undisturbed streams (Table 6). Since this index is sensitive to the level to which samples are classified and to the mesh size of the collection nets, the magnitude of the indices in this study cannot be compared with other studies unless sample classification levels and mesh size are identical.

When macroinvertebrate densities were examined at the order level, they were not always greater for the logged sites (Fig. 2). Caddisflies, beetles, and true flies were significantly more numerous at logged sites than at paired undisturbed sites (Wilcoxon signed rank test), while differences for mayflies, stoneflies, and midges were not significant.

Macroinvertebrate density varied considerably with elevation (Fig. 3). The water at the higher elevation sites was probably colder than at the lower elevation sites. Colder water might have depressed insect populations and their food supplies. Porter and Meehan (1987) showed that for a northeastern Oregon stream (and a 280-mm net mesh size), macroinvertebrate numbers increased 2.6 times from spring to summer, a time when stream temperature was rising. Similarly, McHenry (1986) found that stream segments in New Mexico at higher elevations and with steeper stream gradients had fewer macroinvertebrates than streams at lower elevations and with lower stream gradients.

Regression analysis shows that differences in elevation and stream shading account for much of the variability in total macroinvertebrate density among undisturbed sites (Fig. 3). Figure 3 also includes the logged sites, three of which have unusually high values for macroinvertebrates considering their elevation and angular canopy density.

Cattle grazing can increase levels of nitrogen, phosphorus (Doran et al. 1981; Schepers and Francis 1982), and carbon (Owens et al. 1983) in streams flowing through pastures. However, the effect of this enrichment on the macroinvertebrate community has not been documented. We explored the possibility that macroinvertebrate levels were elevated at logged sites because of heavier grazing by cattle. Each site was assigned a grazing intensity (low, medium, high) based on how much the cattle had disturbed riparian grasses and forbs by late summer. An analysis of variance with elevation, square root of elevation, and angular canopy as covariates showed that grazing intensity was not a significant factor.

The elevated macroinvertebrate levels at some logged sites may be related to the type of shade provided by streamside vegetation. Logged sites get more of their shade from streamside brush and less from conifers. The non-uniform and somewhat translucent shadow cast by brush may allow more solar energy to reach the stream channel, thereby increasing aquatic productivity.

Prior to 1989 we had no water temperature data for streams in the study area and could not test the hypothesis that harvesting next to stream channels resulted in water temperature increases. However, stream temperature measurements taken before and after a wildfire that began in late July, 1989 provided us with some useful information. The wildfire consumed one-third of the Big Sheep Creek drainage (near sites 3, #4, #5, #6, #7 and 8) and killed much of the riparian vegetation. A

TABLE 6. Macroinvertebrate numbers.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Paired sites[a]	a	a	b	b	c	c	d	d	—	—	—	—	—	—	e	e
Riparian stand[b]	L	U	L	U	L	U	L	U	U	U	U	U	U	U	L	U
Elevation (m)	1400	1190	1220	1310	1490	1550	1520	1520	1550	1430	1070	1130	1310	1100	945	975
Trichoptera [caddisflies] (#/sample)	134	56	90	36	54	40	67	47	16	33	46	33	32	57	151	98
Coleoptera [beetles] (#/sample)	71	36	236	104	39	10	33	4	0	2	162	50	10	26	107	62
Ephemeroptera [mayflies] (#/sample)	76	197	138	159	123	55	211	120	172	111	235	172	96	219	423	200
Plecoptera [stoneflies] (#/sample)	12	22	22	29	21	14	20	30	21	17	72	34	42	63	83	122
Diptera[c] [true flies] (#/sample)	43	5	24	23	16	8	10	9	2	2	49	7	4	12	25	7
Chironomidae [midges] (#/sample)	94	43	12	67	60	20	76	31	13	15	169	91	19	50	174	132
Total — Mean	430	359	522	418	313	147	417	241	224	180	733	387	203	427	963	621
— SD[d]	71	45	218	141	126	42	232	93	56	35	147	233	203	135	283	300
Diversity index[e]	3.9	3.7	3.8	3.9	4.0	4.3	3.9	4.2	3.2	3.8	3.7	3.5	4.2	4.0	3.6	3.9

[a]Sites with the same letter are paired.
[b]L = logged site, U = undisturbed site.
[c]Excluding Chironomidae.
[d]Standard deviation of five samples at each site.
[e]Shannon-Weiner Diversity Index on lowest operational taxa.

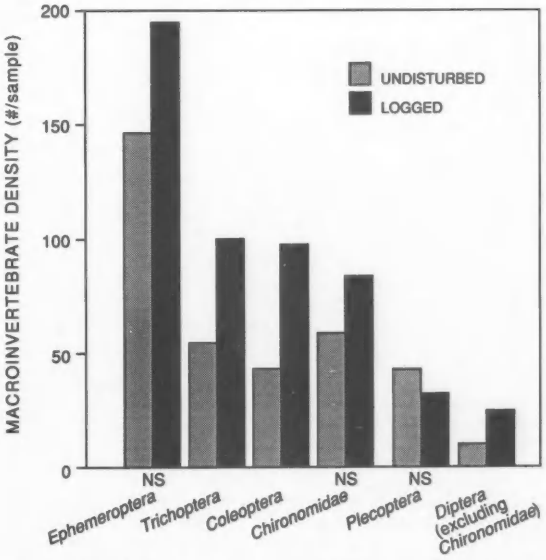

FIG. 2. Macroinvertebrate density for paired logged and undisturbed sites. NS indicates not significantly different at the $p = 0.05$ level according to the Wilcoxon signed ranks test. Total macroinvertebrate numbers average 529 per sample for logged sites and 357 for paired undisturbed sites. The diferrence between the two was significant at the $p = 0.05$ level.

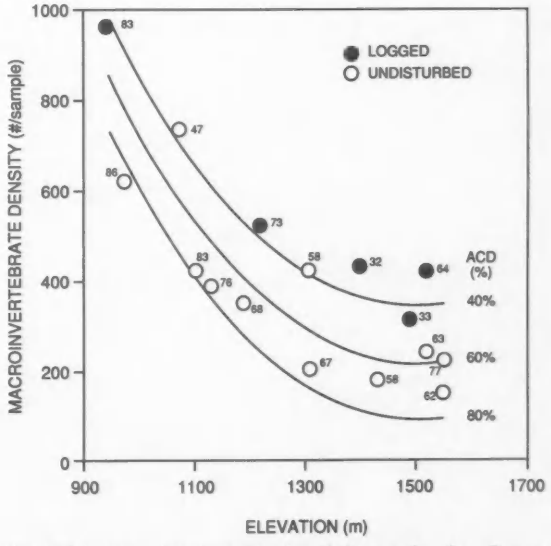

FIG. 3. Macroinvertebrate density in relation to elevation. Percent angular canopy density (ACD) for each site is indicated next to the point. Curved lines based on the regression equation for undisturbed sites: Macroinvertebrate density = 15547 + 9.91 [elevation (m)] − 770 × [elevation$^{0.5}$] − 6.20 [angular canopy density (%)], $R^2 = 0.83$.

thermograph located at a point in the watershed where the drainage area was 58 km^2 recorded maximum temperatures of 18°C during several hot and sunny days before the fire. Maximum temperatures taken at the same location 8 to 15 d after the fire were unchanged despite the loss of brush and conifer needles in the riparian zone of one-third of the watershed. Stream temperatures in this region seem to be insensitive to changes in channel shading. Therefore, the minor changes in stream shading caused by the levels of cutting investigated in this study are not likely to produce increases in stream temperature, even immediately after harvest. Increases in macroinvertebrate numbers following logging are probably a result of increased exposure to sunlight rather than to increased temperature.

Conclusions

The number of deep pools that remain during summer low flow which also do not freeze during winter is probably an important factor in determining fish carrying capacity for these small streams (P. Boehne, United States Forest Service, LaGrande, OR, unpubl. data). Timber harvesting did not alter levels of the woody debris essential for creating most pools within these streams. Timber harvesting increased macroinvertebrate numbers without altering diversity. However, increases in food supply are likely to result in increases in fish productivity if other habitat features are not degraded. Some factors that could have had a harmful effect on some fish were not evaluated in this study. One of these factors was subsurface substrate composition. Judging from the low natural sediment yields of these streams and the lack of obvious sources of sediment from logging activities, subsurface substrate composition is probably unaffected by logging.

The wide variation in channel features and aquatic insect levels found even among sites with undisturbed riparian stands illustrates the importance of considering all aspects of a watershed before deciding about its inherent capacity for fish production. For example, adding logs to the channel of a stream to create more pools and increase fish productivity might not be successful if the stream is too steep to form many pools or if the elevation is so high that suppressed macroinvertebrate levels limit fish productivity.

Residual live trees and snags in logged riparian zones will continue to add woody debris to stream channels in the decades ahead. Existing debris, especially the rot-resistant larch logs, will continue to influence pool abundance in the streams for years. At sites where heavy cutting has occurred on only one side of the stream, trees on the other side will be the primary source of woody debris in the future and should be managed accordingly.

Nature's disturbances need to be taken into account when planning for the protection of fisheries resources. Following fire or insect infestation, large streamside trees (especially larch and Douglas-fir) provide the large pieces of debris that can persist for decades until a new stand is established and is old enough to provide debris. Extensive salvage logging of the large logs near the stream may leave the stream deficient of pool-forming debris at some time in the future. Care is also needed to leave a mixture of tree species to increase the chances that some riparian trees will survive insect attacks. For example, at site 7 the original stand was a mixture of spruce, larch, and Douglas-fir. Logging removed nearly all the larch and Douglas-fir. The residual stand of spruce was killed by bark beetles and now few live trees remain. In this case, leaving some of the larch and Douglas-fir within the riparian stand could have better ensured a long-term supply of woody debris.

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Reduced Catch of Under-Sized Cod (*Gadus morhua*) in Longlining by Using Artificial Bait

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Løkkeborg, S. 1990. Reduced catch of under-sized cod (*Gadus morhua*) in longlining by using artificial bait. Can. J. Fish. Aquat. Sci. 47: 1112–1115.

Three sizes of artificial bait incorporating shrimp flavour into polyurethane foam were compared with natural shrimp bait of the size used in commercial longlining. Fishing trials were conducted during the spring fishery for Atlantic cod (*Gadus morhua*) off the coast of Northern Norway. The small artificial bait gave almost the same catch rate as natural shrimp bait, whereas artificial baits of larger sizes compared poorly. The length distribution of the catch was influenced by both bait type and bait size. Fewer small cod were caught on the artificial baits. There was no difference in the number of large cod. Mean cod length was therefore higher on the artificial baits and increasing the artificial bait size accentuated the difference. These effects are explained by different preferences with regard to prey species and size between small and large cod. The size selective effects of bait type and bait size are discussed in relation to conservation aspects.

Des appâts artificiels en mousse de polyuréthane à saveur de crevette de trois tailles différentes ont été comparé aux appâts de crevette naturelle de la taille utilisée dans la pêche à la palangre commerciale. Des essais de pêche ont été effectués pendant la pêche printanière à la morue (*Gadus morhua*) au large de la côte nord de la Norvège. Les petits appâts artificiels ont donné à peu près le même taux de capture que les appâts naturels; les appâts artificiels plus gros ont donné des résultats beaucoup moins satisfaisants. La distribution des captures sur la longueur était influencée à la fois par le type de l'appât et par sa taille. Un nombre moins élevé de petites morues ont été capturées avec des appâts artificiels. On n'a relevé aucune différence dans le nombre de grosses morues. La longueur moyenne des morues était par conséquent supérieure dans la pêche avec appât artificiel et plus la taille de celui-ci augmentait plus la différence était prononcée. Ces effets sont expliqués par les différences de préférences en matière d'espèces proies et de taille des proies entre les petites et les grosses morues. Les effets sélectifs du type et de la taille de l'appât sur la taille des poissons sont analysés en fonction des divers aspects de la conservation.

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Models of the dynamics of fish populations and examples of mismanagement and overexploitation of fish stocks have demonstrated the importance of protecting young, immature fish to achieve the basic fishery management objective of maximum sustainable yield (Gulland 1977; Beverton 1984). Optimizing the long-term yield of a fish resource therefore requires development of and exploitation by size selective fishing methods. A prerequisite for this is an improved understanding of the interaction of the resource with the fishing gear.

Studies on how the configuration of gear affects the size of fish harvested have mostly focused on trawl and gillnet (Anon 1963; Pope et al. 1975). Few investigations concerning selection by line fishing have been published (Ralston 1982), although hook and bait size are regarded as the most important factors affecting the size of fish caught (Pope 1966). However, type and shape of bait may also prove to be of importance.

Longline fishing trials for Atlantic cod (*Gadus morhua*) showed that larger hooks baited with larger baits caught fewer small fish than smaller hooks baited with smaller baits, whereas there was no difference for large cod (McCracken 1963). Johannessen (1983) investigated the independent effects of bait size and hook size, and concluded that the major size selective effect was due to the bait size. The hook size affected catching efficiency rather than size selectivity; smaller hooks caught more cod of all sizes than larger hooks. However, in a handline fishery for other species, Bertrand (1988) obtained no effect on size selectivity by hook size or bait size.

The size and shape of natural bait are restricted by the geometry of the bait species, especially for baits of whole specimens (e.g. shrimp, anchovy, capelin). An artificial bait can, however, without limitation be moulded in various shapes and cut into appropriate sizes. This gives a unique chance of studying the size selectivity of bait fishing.

As part of a project to develop artificial bait, the catching efficiency and size selectivity of a shrimp-flavoured artificial bait were tested in comparison with natural shrimp bait. The present paper describes how the shape and size of the artificial bait affected the size of cod caught.

Materials and Methods

Baits

The artificial baits were provided by Whitney Marine Laboratory, University of Florida. The baits were composed of a reinforced polyurethane foam impregnated with feeding attractants that occur in natural shrimp bait. The rectangular

TABLE 1. Weights and sizes of artificial bait of three different size groups measured after 2–3 d soaking. Means for 25 baits.

Size group of artificial bait	Weight[a] (g)	Size (mm)
Large[b]	20.1 (1.5)	61 × 24 × 14
Large	15.6 (1.6)	56 × 22 × 15
Medium	8.0 (1.1)	39 × 24 × 9
Small	2.8 (0.6)	35 × 21 × 4

[a]Standard deviation in brackets.
[b]Bait tested in April 1987; the other baits were tested in April 1988.

TABLE 2. Gear parameters for the monofilament longline.

Mainline	– Material:	Polyamide monofilament
	– Dimension:	Diam. 2.0 mm, length per line 750 m
Snood	– Material:	Polyamide monofilament
	– Dimension:	Diam. 0.8 mm, length 70 or 90 cm
	– Mounting:	Swivel
Hook	– Type:	Mustad Wide Gap, qual. 72940, no. 5/0
	– Number:	288 per line, hook spacing 2.5 m

foam was cut into three different sizes; small (35 × 20 × 2 mm), medium (35 × 20 × 5 mm) and large (50 × 20 × 8 mm). The concentration of attractants in each bait was normalized so that each bait released the same amount of attractants irrespective of size. The baits swell when soaked in water and the real sizes when fishing are given in Table 1.

The natural bait was shrimp (*Pandalus borealis* Krøyer) of one size (mean 79 × 33 × 14 mm for 25 shrimps), measured as the length to the end of the rostrum, the maximum depth and the maximum width in the dorsoventral plane, respectively. Mean weight was 11.2 g (SD = 1.8). This is the size of shrimp used in commercial fishing. The natural bait was therefore larger than the artificial baits and differed from these also in shape.

Fishing Ground and Gear

The fishing trials were conducted from April 23 to 30, 1987, and from April 22 to 30, 1988, on a commercial longliner operating about 20 nautical miles off the coast of Finnmark (Northern Norway) at 145–285 m depth. In 1987, only the large artificial bait was tested against natural bait.

A semi-pelagic monofilament longline soaked for 2 or 3 d and floating 2–4 m above bottom was used. Gear parameters are specified in Table 2.

Experimental Design

Each experiment was based on paired comparison between one size of artificial bait and natural shrimp bait. The experimental longlines were baited with the two baits in clusters of 48 similarly baited hooks. In each comparison 5–13 longlines with a total of 1440 to 3744 hooks were set. During hauling of the gear the condition of each hook (species of hooked fish, loss of bait, entanglement, loss of hook) and the total length of each cod caught were recorded on a portable data terminal (Micronic 445L, Floen 1985).

Results

The total catch rate ranged from 20 to 43 fish per 100 shrimp-baited hooks. The most abundant species was Atlantic cod (*Gadus morhua*), which in the different bait comparisons accounted for 40–69% of the total catch. Species such as haddock (*Melanogrammus aeglefinus*), torsk (*Brosme brosme*) and redfish (*Sebastes marinus*) were taken in numbers too low for analyses of size selection.

The small artificial bait gave nearly the same catch rate as natural shrimp bait, whereas artificial baits of larger sizes compared inferiorly with the shrimp bait (Table 3). In all comparisons artificial baits caught a lower number of small cod, whereas there was no difference in the number of large cod (Fig. 1). Artificial baits therefore caught cod of higher mean

TABLE 3. Numbers and mean lengths of cod caught on natural shrimp bait and artificial bait of three different sizes.

Type of bait	Hooks fished[a]	Number caught	Catch per 100 hooks[b]	Number undersized[c]	Mean length[d] (SD)
Shrimp bait[e]	1716	372	21.7	8 (33)	59.7 (9.2)
			**		**
Artificial bait, large[e]	1577	252	16.0	2 (7)	63.8 (9.2)
Shrimp bait	704	158	22.4	4 (19)	57.5 (13.2)
	670	59	**	0 (3)	*
Artificial bait, large			8.8		61.5 (15.3)
Shrimp bait	1383	179	12.9	10 (33)	55.1 (12.7)
			**		*
Artificial bait, medium	1332	62	4.7	1 (7)	58.4 (12.5)
Shrimp bait	711	145	20.4	4 (20)	62.7 (16.6)
Artificial bait, small	693	130	18.8	0 (6)	64.1 (14.2)

[a]Number of hooks recorded during hauling excluding entangled and lost hooks.
[b]Differences in catch rate were tested by binomial test (**$p<0.001$).
[c]Less than 42 cm total length and less than 47 cm total length for numbers in brackets.
[d]Differences in mean cod length were tested by two-sample t-test (*$0.05<p<0.10$, **$p<0.001$).
[e]Results from April 1987; the other results are from April 1988.

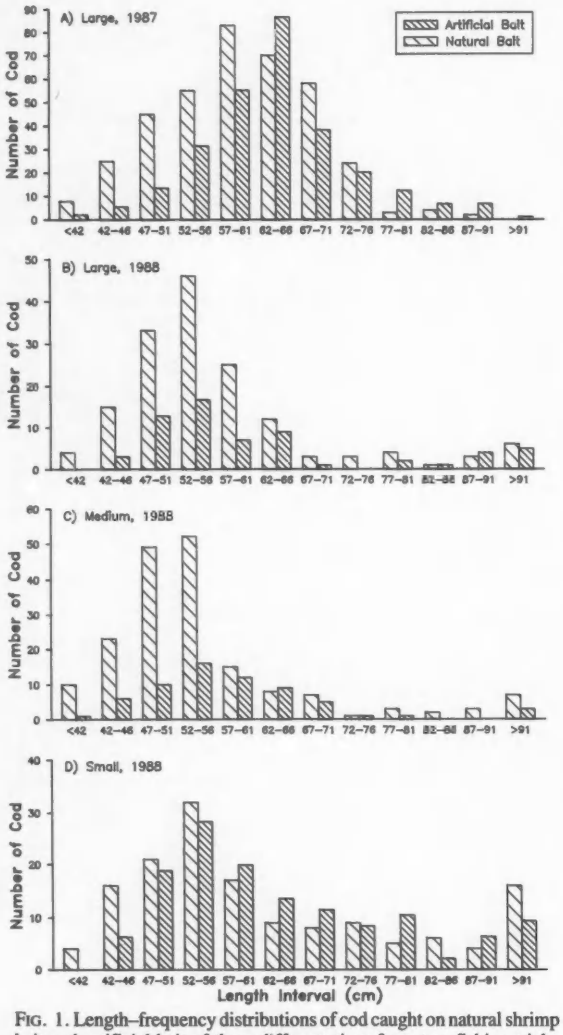

FIG. 1. Length–frequency distributions of cod caught on natural shrimp bait and artificial bait of three different sizes from two fishing trials; April 1987 (A) and April 1988 (B, C, and D).

length than shrimp bait (significant at 5% level only for large bait, Table 3). This difference in mean cod length increased with increasing size of the artificial baits, from 1.4 cm for the small bait to about 4.0 cm for the large bait.

The proportion of under-sized cod (<42 cm) caught on artificial baits, pooled across bait sizes, was significantly lower than for natural shrimp bait (0.6 and 3.0%, respectively; binomial test, $p<0.001$). The artificial baits also caught a lower proportion of cod below 47 cm (see last paragraph of Discussion) than natural bait (4.6 and 12.3%, respectively; $p<0.001$).

Discussion

Results indicated that all three size groups of artificial bait caught, on average, larger cod than natural shrimp bait. The difference was caused by a lower proportion of small fish in the catches taken by artificial baits. This difference cannot be explained by the effect of absolute bait size. Larger baits have been shown to catch less small cod than smaller baits (McCracken 1963; Johannessen 1983). The artificial baits were, however, smaller than the shrimp bait, and a prevailing effect of bait size should therefore have given a result contrary to the observed. As the size of the shrimp was considered the maximum length, depth, and width, the shrimp might have appeared somewhat smaller to the cod than this maximum size, but not smaller than the small- and medium-sized artificial baits which were only half as long.

The preference by smaller cod for the shrimp bait may therefore be based on discrimination between the different shapes of natural and artificial baits. Mehl et al. (1985) found that a general trend in the diet of the Barents Sea cod was that shrimp was the dominant prey of smaller cod, whereas fish were the major prey category of larger cod. The small cod's preference for shrimp over another type of prey may result from a long-term exposure or possibly a "search image" formation (see Dawkins 1971; Krebs 1973). The "search image" phenomenon implies that a foraging animal bears an image of the object sought, attending only to certain diagnostic clues which are used in the search (Hart 1986).

The tendency for smaller cod to be caught less often by the artificial baits than by the shrimp bait may also reflect a restrained response towards attacking a novel prey. Stickleback (*Gasterosteus aculeatus*) confronted with a new prey species first started to eat the prey after 50–200 encounters (Beukema 1968), and naive rainbow trout (*Oncorhynchus mykiss*) required several days of exposure before they approached novel prey (Ware 1971). In a field study of cod only 5% of the fish responded to mackerel bait which can be regarded as an unfamiliar prey (Løkkeborg et al. 1989). Such restraint in the feeding behaviour might, in the present study, have been less pronounced in larger cod. Larger cod have a greater diet breadth (Palsson 1981; Mehl et al. 1985) and therefore have greater experience of edible prey types.

Furthermore, the lower number of small cod caught by artificial baits may be explained by differences in texture and taste. Even if a predator responds to the smell of a prey that releases feeding attractants of shrimp, the predator may reject the prey if its texture and taste differ from that of shrimp. Especially the texture of the foam of the artificial baits differs from natural shrimp. Also this effect might have been less pronounced in larger cod because of their greater breadth of diet.

Differences in bait shape and possibly texture rather than size are thus the most likely explanation for the lower proportion of small cod in the catches on artificial baits. However, the observed increase in mean cod length with increasing size of artificial baits demonstrated that bait size also affects size selectivity. Fishing trials with natural bait of various sizes showed the same effect (Johannessen 1983). These findings may reflect an optimal relationship between predator size and the size of its prey. Most field studies of prey preference by fish show selection for certain sizes (Hart 1986), and Werner (1974) found that optimal prey size was linearly related to predator size.

The use of passive fishing gear such as longlines is being encouraged by fisheries policy for, among other things, their greater selectivity concerning size and species of fish harvested (Sutterlin et al. 1982). By its low exploitation of the younger size groups, longline is a more conservation fishing method than trawling (Bjordal 1988). The present results indicate that the size selectivity of this gear could be further improved by using baits of certain shapes and sizes.

These properties are of particular importance when harvesting an overexploited fish stock with a high proportion of small, immature fish. This is the present situation for the northeast Arctic cod stock, and has lead to the suggestion of increasing the legal size of cod which may be landed to 47 cm (International Council for the Exploration of the Sea; Directorate of Fisheries, Bergen). This legislation was put into practice at the beginning of 1990. The proportion of under-sized fish in the total catch of a commercial longliner may therefore, as indicated by the present result, become above the legal proportion of 15%. This problem could be solved by the introduction of a more size selective bait.

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Variation in the Competitive Abilities of Fishermen and its Influence on the Spatial Distribution of the British Columbia Salmon Troll Fleet

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Abrahams, M. V., and M. C. Healey. 1990. Variation in the competitive abilities of fishermen and its influence on the spatial distribution of the British Columbia salmon troll fleet. Can. J. Fish. Aquat. Sci. 47: 1116–1121.

We estimated the catch rates of individual fishing vessels within the British Columbia salmon troll fleet from the data of a 7-yr log book program. Catch rates varied considerably among vessels. A portion of the variation could be attributed to environmental variation. However, there were also significant differences in competitive ability among vessels. The top ranked vessels had a catch rate 3.6 times that of the lowest ranked vessels. Top ranked vessels distributed their fishing effort among more fishing areas than bottom ranked vessels, but were also more successful at catching fish when fishing in the same areas as bottom ranked vessels. This information, through an application of the ideal free distribution theory, can be used to develop a model that will describe the expected relationship between vessel distribution, vessel catch rate, and fish distribution, potentially allowing vessel distributions to be used as a tool for assessing fish stocks.

Nous avons évalué les taux de capture de chaque navire de la flotte de pêche à la ligne traînante au saumon en Colombie-Britannique à partir des données recueillies dans le cadre d'un programme de levé de sept ans. Les taux de capture variaient considérablement entre les divers navires. Une partie de ces variations pourrait être attribuée aux variations du milieu. Toutefois, on a également noté d'importantes différences dans la capacité concurrentielle des navires. Le taux de capture des meilleurs navires était 3.6 fois plus élevé que celui des navires en bas de liste. L'effort de pêche des premiers était réparti sur une plus vaste superficie que les seconds et leurs captures étaient également plus importantes lorsqu'ils pêchaient dans les mêmes secteurs. En appliquant la théorie de la distribution libre idéale, ces renseignements peuvent servir à mettre au point un modèle pour décrire le rapport prévu entre la distribution des navires, leurs taux de capture, et la distribution des poissons, et permettre éventuellement d'évaluer les stocks de poissons à partir de la distribution des navires.

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Studies on the fleet dynamics of commercial fishing vessels have traditionally viewed the problem from a population perspective. Typically, questions of interest include factors which affect the size of (or investment in) the fleet, such as changes in the abundance of prey species and their economic value (Botsford et al. 1983; Gatto et al. 1975; Charles 1983). Recently, there has been growing interest in examining strategies adopted by fleets for exploiting their resource. These include strategies used for finding fish (Mangel 1982; Mangel and Clark 1983), and movement patterns of fishing vessels (Hilborn and Ledbetter 1979). Few studies have considered the consequences of variation in catch rate on the spatial distribution of the fleet (but see Hilborn and Ledbetter 1979). This study is a detailed examination of the catch rates of a group of trollers and the consequences of catch rates on the spatial distribution of the west coast troll fleet through an application of the ideal free distribution theory (IFD).

Fretwell and Lucas (1970) and Fretwell (1972) introduced the IFD as a theoretical model to explain the distribution of animals with respect to their resources. For animals to distribute themselves in accordance with an IFD, they must satisfy two assumptions. First, all animals must have perfect information about the distribution of resources in their environment (i.e. they are ideal). Second, all individuals should be equal in their competitive abilities such that they have 'free' access to resources anywhere in their environment. When animals conform to these assumptions, they will distribute themselves so that they all receive equal amounts of the resource, regardless of where they occur.

Fretwell (1972) recognized that these assumptions were unlikely to be true. However, deviations from this model can identify factors important in determining the spatial distribution of animals. Recently, a number of studies have examined the spatial distribution of predators with respect to their prey (see Abrahams 1986 for a review). In the majority of these studies, predators tended to overuse poor patches (areas with a below-average proportion of resources) and underuse good patches (areas with an above-average proportion of resources). In many cases, the authors of these papers argued that their animals were not all of equal competitive ability and that this was the most likely reason their results did not comform exactly to an IFD.

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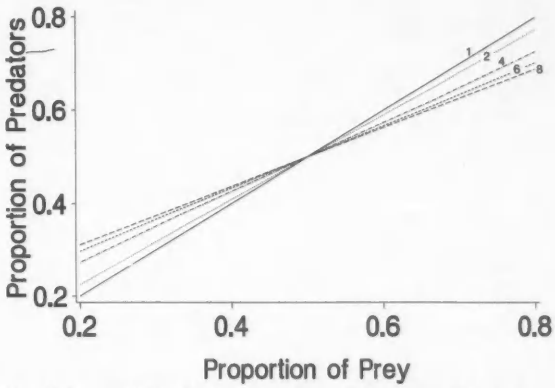

FIG. 1. Expected distribution of predators with respect to their prey as the range of competitive abilities changes (ratio of the best competitor to the worst competitor). Numbers on each line represent the ratio of competitive abilities. (From Houston and McNamara 1988).

Parker and Sutherland (1986) and Sutherland and Parker (1985) suggested that when animals differ in competitive ability, the sum of competitive abilities rather than the animals themselves will be distributed in accordance with an IFD. There are a number of numerical distributions of animals relative to their resources that will satisfy Parker and Sutherland's model, but Houston and McNamara (1988) have demonstrated that overuse of poor patches is statistically the most likely distribution. Furthermore, increasing the difference in competitive abilities, or the number of competitors, or both will increase the magnitude of this effect (Houston and McNamara 1988, Fig. 1).

Fishermen are faced with a patch choice problem in their search for fish. Although the ocean appears to be a homogenous environment (at least at the surface), fish are known to aggregate in particular areas. For salmon trollers, aggregations of salmon are typically found in upwelling areas associated with steep slopes on the continental shelf and, thus, are identifiable from navigational charts (Healey et al. 1990).

That trollers recognize and focus on patches of salmon is evident from the spatial distribution of the troll fleet off the west coast of Vancouver Island (Fig. 2). In this paper we examine characteristics of trollers to determine the extent to which they deviate from the assumption of equal competitive ability. This information can then be used to describe the extent to which the troll fleet should deviate from an IFD and thus the predicted distribution of the troll fleet relative to the distribution of fish.

Characteristics of Troll Vessels in the Salmon Fishery

Troll vessels off the coast of British Columbia fish for salmon (*Oncorhynchus* sp.) by hook and line, using six lines baited with lures and flashers placed at different depths. Within the fleet there are three classes of vessels: day boats, ice boats, and freezer boats. The majority of the vessels in the fleet (and in this study) are ice boats, which preserve their catch with ice, and undertake fishing trips no longer than 2 wk in duration. Day boats also preserve their fish with ice, but return to port daily to sell their catch. Freezer boats freeze their catch, "glazing" them in ice for preservation. They are the largest and most sophisticated in the fleet and need only return to port when they have a full load of fish or require supplies, allowing them to fish continuously for periods in excess of 60 d. Typically trollers own and operate their own vessel with the assistance of a hired deck hand.

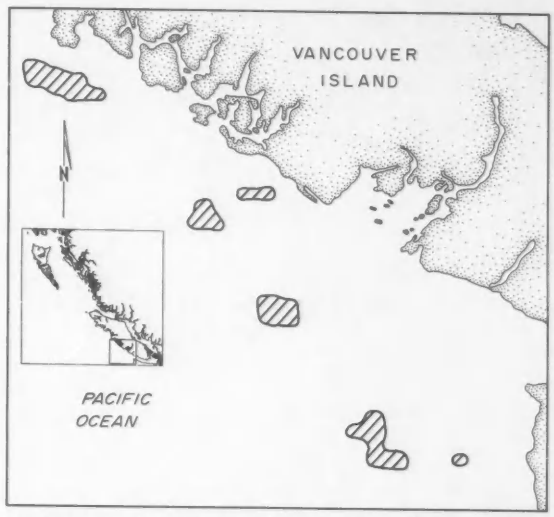

FIG. 2. Observed aggregations of trollers off southwest Vancouver Island July 28, 1982. Observation from Tofino traffic control radar. Minimum vessel density within aggregations = 0.105·boats·km^{-2}. Inset shows British Columbia coast.

Methods

The Canadian Department of Fisheries and Oceans has maintained data for the British Columbia coast (except for the Strait of Georgia) on daily catch of salmon by species from individual troll boats during the years 1981 to 1987. Twenty-eight, 20, 26, 70, 82, 79, and 86 vessels provided data in 1981 to 1987, respectively (Healey 1986, Jordan and Carter 1987).

The vessels participating in this program caught six different species of Pacific salmon: chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye (*O. nerka*), and steelhead (*O. mykiss*). As these fish vary considerably in both size and value, catch composition differed among vessels, and we converted the catch data for each boat to a dollar value. To do this, the weight of the fish was assumed equal to the average weight of each species landed in each statistical area each week (Canada Department of Fisheries and Oceans, unpubl. data). Dollar value of the catch was then: $V = \Sigma W_i N_i P_i$ where W_i = average weight of species i, N_i = number of species i caught, P_i = price of species i.

We assumed that the goal of each skipper was to maximize the rate at which he made money while fishing. If this is true, then competitive ability can be represented by the short-term rate of accumulating income. We calculated a daily estimate of each vessel's competitive ability by dividing the total cash value of a daily catch by the number of hours spent fishing. This estimate of competitive ability is independent of the total amount of money each vessel earned.

Results

Variation in Catch Rates Among Years

Over the 7 yr of this study, the price of each salmon species changed in both absolute and relative terms (Fig. 3). From 1981

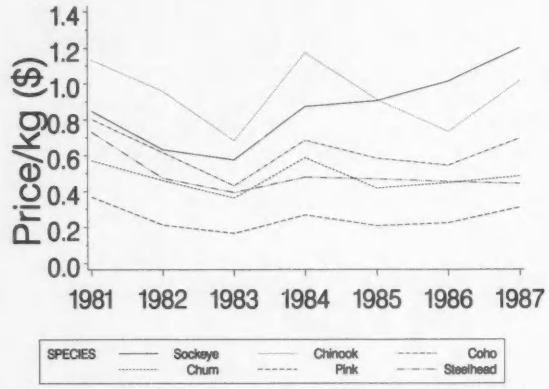

FIG. 3. Average annual price (in 1981 dollars) for six species of pacific salmon from 1981 to 1987.

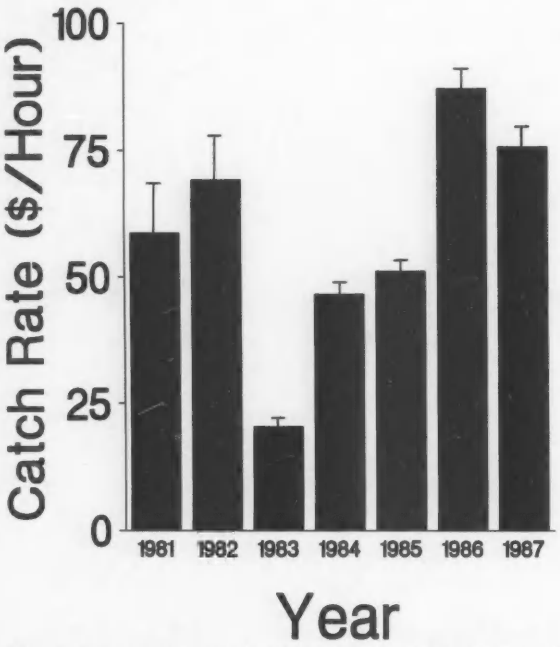

FIG. 4. Average catch rates (in 1981 dollars) for July and August of each year. Error bars represent one standard error.

to 1984, chinook salmon commanded the highest price/kilogram, and their price/kilogram increased with their size. During 1986 and 1987, however, sockeye salmon commanded the highest price/kilogram and their value relative to the other species increascd from 1983 to 1987.

Between 1981 and 1987, regulations changed to restrict the fishing season. Details of these regulatory changes, which were implemented to conserve declining stocks of chinook salmon and to meet terms of the Canada/United States salmon interception agreement are given in Healey (1986); Argue et al. (1987); and Shardlow et al. (1986, 1988). In relation to the analyses we planned to perform, the most significant changes in regulations were a gradual elimination of the spring fishing season for chinook salmon and earlier closure of the troll fishery in September. The fishery was gradually restricted to the months of July and August. Although these regulation changes cut the annual fishing time by more than half, most of the catch was historically taken during July and August. In addition, in 1985 and thereafter, catch ceilings were imposed on all species on the west coast of Vancouver Island. We therefore restricted our analysis to log book records from July and August each year.

Catch rates (1981 dollars/hour) during these summer months varied considerably between years (multifactor ANOVA, $F_{6,2683} = 58.6$, $P = 0.0001$); the catch rate of 1986 being almost four times that recorded in 1983 (Fig. 4). The range of variation exceeded that which could be accounted for by changes in the value of salmon and are likely due to changes in the abundance of fish (Healey 1986).

Within Year Variation

When catch data were grouped by week, there was a significant effect of week on catch rates (multifactor ANOVA, $F_{9,2683} = 54.7$, $P = 0.0001$). Catch rates were highest in the mid and late summer and were due to pink and sockeye salmon becoming available to the trollers at this time of the year whereas earlier only chinook and coho were available (Healey 1986).

Variation in Catch Rates Among Areas

There was also a significant effect of fishing area on the reported catch rates for all years (multifactor ANOVA, $F_{19,2683} = 30.9$, $P = 0.0001$). Unlike the within year variation, this effect was less consistent (and less predictable) among years. Fishing areas that had catch rates significantly higher than all others ranged from southern to northern British Columbia in different years.

Variation in Catch Rates Among Vessels

There was high variation in catch rate among vessels within a year (multifactor ANOVA, $F_{128,\ 2683} = 10.15$, $p < 0.0001$). This variation should reflect competitive ability, but it was also influenced by the temporal and spatial variability in the availability of fish noted above. There may be no real difference in competitive ability among fishing vessels, and therefore the variation in catch rates may simply be due to some boats being in the right place at the right time. To determine whether variation in catch rates among vessels was at least partly due to differences in competitive ability we compared the observed frequency distribution of catch rates to a null distribution in which all variation was environmentally determined.

We transformed catch rates for each vessel for each year to the number of standard deviates from the mean for all vessels. This number represented each vessel's relative competitive ability, independent of the absolute catch rate for that year. From these data we calculated an average catch rate for each vessel that provided three or more years of data (73 of the 141 different vessels in this program). The frequency distribution of these average catch rates was assumed to represent the range of competitive abilities within the fleet.

We constructed the null distribution using all the observed data. Our fundamental assumption was that no differences existed in competitive ability so that the range of catch rates observed each year was generated solely by environmental variation. To create this distribution new catch rate values were assigned to each of the 73 vessels by randomly drawing (with replacement) one observation from the observed data for all

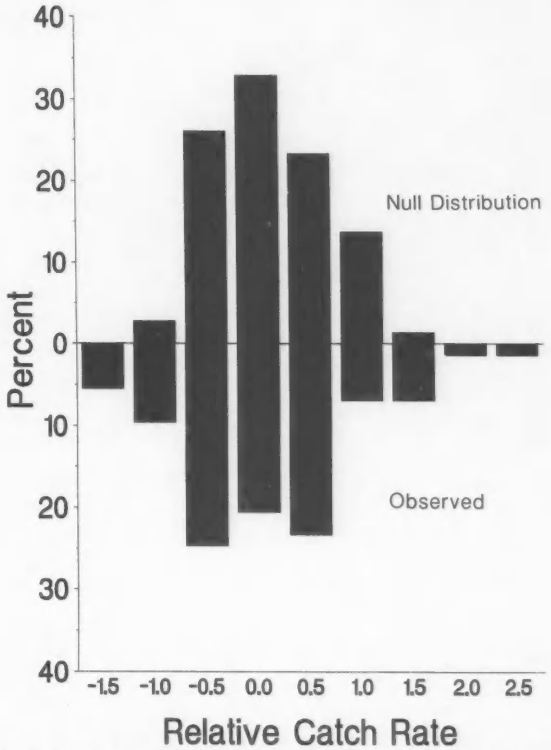

FIG. 5. Observed and predicted distribution of average relative catch rates (catch rates as unit normal deviates). Predicted model (null distribution) assumes there are no differences in the competitive abilities of fishing vessels.

vessels for each year this vessel contributed data. For example, if vessel 1 contributed data in 1981, 1983, and 1986 then a new catch for 1981 was assigned to this vessel from the set of catch rates for all vessels in 1981, and similarly for 1983 and 1986. The average catch rates calculated from these assigned numbers were taken to represent a catch rate distribution due to chance encounters with environmental factors (temporal and spatial) alone. The frequency distribution of these new average values was the null distribution.

If there are real differences in competitive ability among trollers the observed distribution of catch rates should be significantly broader than the null distribution. The two distributions are illustrated in Fig. 5, and are significantly different ($\chi^2 = 50.67, P<0.001$). The null distribution shows much less variation in catch rates than the observed data, indicating that environmental variation alone cannot account for the observed range of catch rates in the troll fleet, and real differences in competitive abilities must exist.

We selected the top five and bottom five ranked vessels from the observed distribution as representative of the range of competitive abilities of the vessels sampled and examined their absolute rates of catch and distribution of fishing effort. All of the vessels in this sample reported catch rates for 1985 through 1987. During this time period, the average catch rate of the top five vessels was 3.6 times that of the bottom vessels. Furthermore, this ratio was 3.5 for those instances in which top and bottom ranked vessels were fishing in the same area at the same time, suggesting that the ratio of catch rates represents a difference in the ability to catch fish, not a difference in the ability to find fish.

The top and bottom ranked vessels differed in the proportion of their time spent fishing in different statistical areas (Fig. 6a, b). All the top vessels fished in a number of different areas. This was in striking contrast to the bottom vessels (Fig. 6b) which spend more than 75% of their time in a single or two adjacent areas, only occasionally fishing in other locations.

To determine whether the fishing patterns of different vessels were a major determinant of fishing success, we described the diversity of where the boats fished by the Shannon-Wiener diversity index. The Shannon-Wiener diversity index is derived from information theory and is calculated as $\Sigma p_i (\log p_i)$ where p_i is the proportion of time spent fishing in area i. The minimum value occurs if a vessel fishes only in one area. The index increases as vessels distribute their time equally between a large number of areas. We calculated a diversity index for each vessel for each year and averaged these values. As with the previous analysis, we used only vessels that provided three or more years of data. A significant positive correlation existed between the average diversity index and catch rate expressed as a unit normal deviate (Fig. 7, multifactor ANOVA, $F_{1,71} = 17.3$, $P<0.0001$, $r^2 = 0.196$). The diversity index was also positively correlated with the proportion of sockeye in the catch (proportions arcsin square-root transformed, $F_{1,71} = 11.69$, $P = 0.001$, $r^2 = 0.141$), suggesting that a broad distribution of fishing effort may be a strategy to promote the capture of sockeye. A significant, but weak negative correlation existed between the diversity index and the proportion of chinook in the catch (proportions arcsin square-root transformed, $F_{1,71} = 7.75$, $r^2 = -0.098$, $P = 0.007$). No other significant correlations existed for other species, or the diversity of species caught.

Another factor which may contribute to differences in competitive ability is vessel size. Vessel length was significantly positively correlated with catch rate (Fig. 8, $F_{1,71} = 22.1$, $r^2 = 0.237$, $P<0.0001$). Whether larger vessel size is a cause or an effect of higher competitive ability is unclear. However, if mobility is a significant factor contributing to fishing success and competitive ability then possession of a larger vessel probably enhances any competitive advantage that a successful fisherman already has. Vessel class (i.e. ice boat or freezer boat) had no significant influence on relative catch rate (multifactor ANOVA, $F_{1,71} = 1.72$, $P = 0.19$). Presumably, the advantage of operating a freezer boat may be to increase the time available for fishing since they require fewer trips to port.

Discussion

Analysis of catch rates among individuals in the British Columbia salmon troll fleet indicates that boats differ substantially in competitive ability. These differences are in addition to considerable temporal and spatial variation in catch rates. The source of the differences in competitive ability is less clear. Striking differences were observed in the allocation of fishing effort between the top and bottom ranked vessels. Top ranked vessels fished in a large number of areas whereas the bottom ranked vessels tended to concentrate their effort in one or two areas. The relationship between catch rate and dispersion of fishing effort was consistent over all vessels.

Vessel length also was positively correlated with catch rate. However, the mechanism responsible for this correlation is not clear. Some fishermen argue that larger vessels fish better

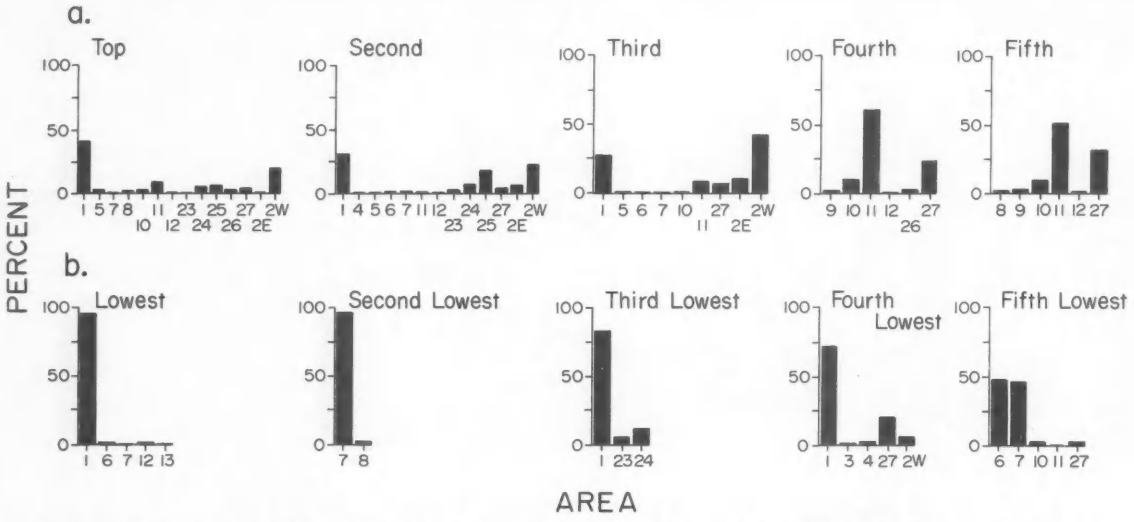

FIG. 6. Percent of time spent fishing in different statistical areas by (a) the top five ranked vessels and, (b) the bottom five ranked vessels.

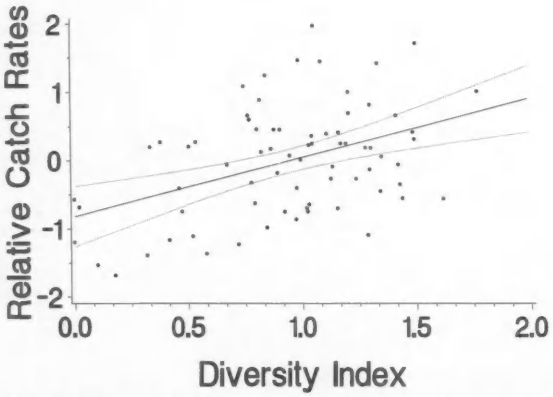

FIG. 7. Relative catch rates as a function of diversity index. Line fitted by least squares (dotted lines are 95% confidence intervals). See text for details.

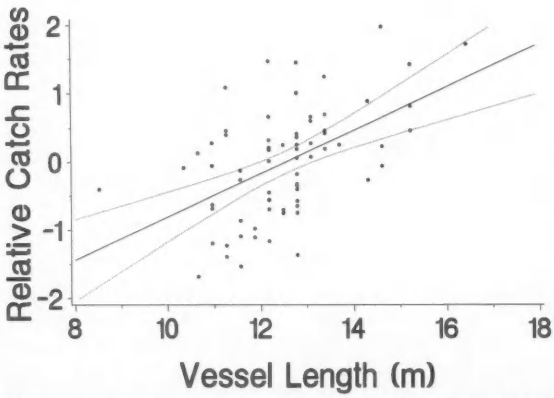

FIG. 8. Relative catch rates as a function of vessel length. Line fitted by least squares (dotted lines are 95% confidence intervals).

because of their greater momentum, allowing them to fish at a constant speed in rough water. A small vessel fishing in the same conditions has its speed slowed by each wave, possibly rendering the gear less effective. Alternatively, only successful fishermen could afford to own large fishing vessels. Therefore, the higher catch rates of the larger vessels may be due to differences in the skills of the skippers, not the vessels per se.

Other factors that may affect competitive ability include characteristics of the vessels such as electrical grounding of the hull, and the type and condition of the equipment on board, as well as the experience and competence of the skipper which would determine the efficiency with which a vessel's equipment was used (e.g. structure and type of lures deployed). Finally, fishing strategies of the skippers may differ in ways other than the way they allocate effort among fishing areas. Evidence that factors such as these are related to competitive ability is provided by a comparison of catch rates among vessels fishing in the same area at the same time. In such a situation, top vessels still did much better than bottom vessels.

Data provided in this study indicate only the gross financial benefits from fishing, not the costs. Vessels with higher catch rates may incur greater financial costs due to movement or hardship due to isolation (see R. Hilborn and R. B. Kennedy, unpubl. data for a discussion of this latter point). Net benefits may be more similar among vessels than indicated by these data but are unlikely to be of a magnitude sufficient to offset our observations (see Figs. 7 and 8).

Hilborn (1985) has suggested that, within the commercial purse seine fishery, two strategies could exist for maximizing the number of fish caught. One strategy is to specialize in a particular area and, by learning everything possible about catching fish in that area, maximize the rate of capture within that area (area specialist). The other strategy is to develop skills appropriate to any area and then to fish opportunistically in a large number of different areas in order to maximize the number of fish captured (movement specialist). In this study, vessels

with the highest catch rates were movement specialists and vessels with the lowest catch rates were area specialists. However, area specialists were not more skilled at fishing in their chosen area than were movement specialists, rather the reverse was true. Nor is there evidence that area and movement specialization exist as discrete strategies within the troll fleet. Rather, these strategies appear to be ends of a continuum. An alternative explanation may be that vessel movement patterns are tied to a skipper's decision about which salmon species to fish for. Increased movement may be necessary if the troller wishes to follow schools of sockeye salmon as they migrate along the British Columbia coast. Area-restricted fishing may reflect a decision to target on chinook salmon. Intermediate movement patterns represent less species specificity in harvesting.

Whatever their underlying causes, absolute differences in competitive ability among vessels will have a profound influence on the distribution of fishing vessels relative to the distribution of fish. As the variation in competitive abilities increases, so does the deviation from an IFD (Houston and McNamara 1988). Therefore, knowledge of the relative competitive abilities of troll fishermen can be used to describe the spatial distribution of the fleet relative to the distribution of available fish. In the case of the troll fishermen, the fleet distribution should be skewed toward regions of lower profitability.

A further benefit of this type of model is that the distribution of fishing vessels may be used to assess the distribution of fish. The distribution of fishing vessels can be mapped from aircraft overflights or from coastal radar installations much more easily than the distribution of fish (Borstad et al. 1982; Healey et al. 1990).

However, if Houston and McNamara's (1988) model is to be used as a stock assessment tool, three problems must be addressed. First, their model assumes that interference (the slope of the relationship between catch rate and vessel density) is -1. Currently, data on this relationship are not available so that this assumption can not be evaluated.

Second, their model assumes that a predator chooses between only two locations. No solution is provided for situations in which the predator has a number of choices. Other studies indicate that the inflection point (i.e. the point at which patches contain fewer predators than prey) should shift towards the origin, tracking the inverse of the number of locations among which the predator is choosing (Abrahams 1986). However, an analytical solution to this question is required before the model could be used in stock assessment.

Finally, a method must be found to convert the relative measures of fish distribution generated by the model to an absolute measure. If the relation between catch rate and vessel density is known, then the catch rate for a single vessel in any patch can be calculated. However, an independent estimate of the relation between fish number and catch rate will be required.

Acknowledgements

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Effect of Copper on the Interaction between the Predator *Didinium nasutum* and its Prey *Paramecium caudatum*

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Doucet, C. M., and E. J. Maly. 1990. Effect of copper on the interaction between the predator *Didinium nasutum* and its prey *Paramecium caudatum*. Can. J. Fish. Aquat. Sci. 47: 1122–1127.

Tests to determine acute copper toxicity levels demonstrated that the protozoan predator *Didinium nasutum* were more susceptible to copper stress than its prey *Paramecium caudatum* Thus we predicted that *Paramecium* and *Didinium* densities, at the equilibrium point of their interaction, would be higher at sublethal copper levels due to a decrease in the predator's efficiency. This situation is likely to produce a decrease in the stability of the system. However, isocline analysis did not support the predictions based on the acute lethality tests. Equilibrium densities of both predator and prey did not change at copper levels between 30 and 180 μg/L. The mathematical analysis suggested that the interaction became less stable with increasing copper concentrations. However, stability decreased due to hormesis in *Didinium* at sublethal copper levels and not due to a reduction in predator efficiency as expected. At 300 μg Cu/L, densities of both species at equilibrium were higher and the stability of the system decreased. This decrease in stability resulted from a reduction in predator efficiency, as 300 μg Cu/L is not sublethal for *Didinium*.

Des essais visant à mesurer les niveaux de toxicité aiguë du cuivre ont montré que le protozoaire prédateur *Didinium nasutum* était plus sensible à une agression par le cuivre que sa proie *Paramecium caudatum*. Nous avons donc estimé que les densités de *Paramecium* et de *Didinium* au point d'équilibre de leur interaction seraient plus élevées pour des doses sublétales de cuivre à cause d'une diminution de l'efficacité du prédateur. Il est probable que cette situation diminue la stabilité du système. Toutefois, l'analyse isocline n'a pas soutenu les prévisions fondées sur les épreuves de létalité aiguë. Les densités à l'équilibre du prédateur et de la proie n'ont pas changé pour des concentrations de cuivre comprises entre 30 et 180 μg/l. D'après l'analyse mathématique, il semble que l'interaction devienne moins stable lorsque les concentrations de cuivre augmentent. Toutefois, la stabilité diminuait à cause de l'hormèse chez *Didinium* pour des doses sublétales de cuivre et non pas à cause d'une réduction de l'efficacité du prédateur comme on s'y attendait. Lorsque la concentration de cuivre atteignait 300 μg/L, la densité des deux espèces à l'équilibre était plus élevée et la stabilité du système diminuait. Cette baisse de stabilité provenait d'une diminution de l'efficacité du prédateur puisqu'une concentration de 300 μg de cuivre par litre ne constitue pas une dose sublétale chez *Didinium*.

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Until recently, single species toxicity tests have provided the majority of information on the effects of toxicants on organisms both in the laboratory and in the field (NRC 1981; Cairns 1983). These tests have provided valuable information on exposure durations and concentrations that can result in changes in survival ability, reproductive success, and physiology of individuals exposed to toxicant stress (Spear and Pierce 1979; Demayo and Taylor 1981; CWQG 1987). However, these tests may not always be appropriate for the examination of the effects of toxicants at the community and ecosystem level (NRC 1981; Cairns 1983). Interactions between species, such as competition and predation, can be disturbed by the presence of toxicants resulting in changes in community structure and dynamics which might be difficult to predict using only toxicological data for individual species (Lawrence and Holoka 1979).

Isocline analysis (Rosenzweig and MacArthur 1963) is a method which enables investigators to predict stability properties of predator–prey interactions. This method has been used to examine the effects of differential predator efficiency (Rosenzweig and MacArthur 1963), food availability (Rosenzweig 1971; Luckinbill 1974), and time lags (Maly 1969) on the stability properties of predator–prey systems. Emlen (1984) reviews the use of graphical models of predator–prey interactions.

In this report we examine the effects of copper on the stability of this protozoan predator–prey system using isocline analysis. Eventually, this method might then be used in combination with single species toxicity data as a significant step towards developing bioassays for communities and ecosystems.

Protozoans are ideal for population and toxicological studies because large populations can be cultured and maintained in the laboratory and high reproductive rates result in many generations in a relatively short period (Honig et al. 1980). Also, interactions between protozoans have been found to be comparable with those observed in metazoans so that inferences about metazoan populations can be made from protozoan studies (Salt 1974; Graham and Canale 1982). In particular, the predator–prey interaction between *Paramecium* and *Didinium*

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has been extensively studied (Luckinbill 1973; Salt 1974; Maly 1978; Hewett 1980, 1988).

Materials and Methods

Culture Methods

Paramecium caudatum (Ward's Biological Supply Company) were cultured in the laboratory in a Cerophyl medium, adjusted to a pH of between 6.8 and 7.0, as described by Bridger (1970) and modified by Sawada (1983). The pH remained within this range for the duration of all experiments. *Didinium* cultures were prepared by adding a small volume of *Didinium nasutum* (Ward's Biological Supply Company) to approximately 250 mL of Cerophyl medium containing *Paramecium*. Cultures containing an abundant food supply for one of the species (*Escherichia coli* for *Paramecium* or *Paramecium* for *Didinium*) are referred to as growth medium throughout the paper. All cultures were maintained under ambient laboratory conditions (approximately 12 h light : 12 h dark at 18°C). An appropriate time frame for experiments was determined by examining the predator–prey interaction for 12, 24, and 48 h. Density changes were relatively low at 12 h whereas at 48 h no *Paramecium* remained. Therefore, all experiments were run for 24 h. All experiments were performed under a 24-h dark photoperiod at 25 ± 0.2°C.

Acute Lethality Tests

The acute lethality of copper to *Paramecium* and *Didinium* was examined using seven copper concentrations (0, 150, 300, 600, 900, 1200, and 1500 μg/L). *Paramecium* or *Didinium* were added to a 50-mL glass beaker containing growth medium. Sufficient food was available for both predator and prey for the duration of all experiments. Copper was added from a stock solution of 12 mg/L Cu^{2+} (prepared from $CuSO_4 \cdot 5H_20$). All copper concentrations were replicated once during each of two blocks over time. A block represents the set of all copper concentrations repeated at a later date. At 4-h intervals, over a 24-h period, a 2.0-mL sample was taken from each beaker and all animals were counted. The final number of animals divided by the initial number of animals was used as an index of survival and reproductive ability. A multiple regression analysis with repeated measures was used to test for significant effects of copper concentration over time (Snedecor and Cochran 1980).

The test concentrations for all further experiments were chosen as 0, 30, 60, 180, and 300 μg Cu/L. These concentrations were nominal and prepared by dilution from a stock solution of 12.30 mg Cu/L (SE = 0.28). The copper concentration of the stock solution was analyzed both before and after experiments by angular flame absorption spectrophotometry (Perkin-Elmer 503 atomic absorption spectrophotometer).

Effect of Copper on *Escherichia coli*

The effect of copper on *E.coli* was tested to ensure that an insufficient food supply for the *Paramecium* was not a factor affecting the interaction between *Paramecium* and *Didinium*. Cultures of *E. coli* at each of the five copper concentrations, diluted $1{:}1 \times 10^6$, were made and plated onto eosine methylene blue (EMB) agar. The plates were examined after a 24-h incubation period at 37°C. A chi-square analysis was used to determine if differences in viable cell density result from the addition of copper.

TABLE 1. Initial densities of *Paramecium* and *Didinium* used in the construction of predator and prey isoclines at various copper concentrations.

[Cu] (μg/L)	Block	*Paramecium* Density (± SE) (#/20 mL)			
0	1	156.4 ± 5.0	77.7 ± 2.5	31.1 ± 1.0	15.5 ± 0.5
	2	57.4 ± 1.7	43.2 ± 1.3	28.7 ± 0.8	14.3 ± 0.4
	3	55.8 ± 1.5	44.6 ± 1.2	33.5 ± 0.9	11.2 ± 0.3
30	1	155.6 ± 7.1	75.4 ± 3.4	33.0 ± 1.5	14.2 ± 0.6
	2	40.4 ± 1.4	30.3 ± 1.0	20.2 ± 0.7	10.1 ± 0.3
	3	45.9 ± 1.5	34.5 ± 1.1	23.0 ± 0.8	11.5 ± 0.4
60	1	177.4 ± 4.0	88.7 ± 2.0	35.4 ± 0.8	17.7 ± 0.4
	2	65.2 ± 2.8	32.6 ± 1.4	16.3 ± 0.7	6.5 ± 0.3
	3	58.8 ± 2.2	35.3 ± 1.3	25.5 ± 0.9	11.8 ± 0.4
180	1	157.0 ± 5.9	78.5 ± 2.9	31.4 ± 1.2	15.7 ± 0.6
	2	59.6 ± 1.6	29.8 ± 0.8	20.9 ± 0.6	8.9 ± 0.2
	3	61.7 ± 3.3	30.8 ± 1.6	20.6 ± 1.1	10.3 ± 0.5
300	1	122.4 ± 5.1	73.4 ± 3.0	48.9 ± 2.0	24.5 ± 1.0
	2	120.9 ± 7.0	100.7 ± 5.8	87.2 ± 5.1	67.1 ± 3.9
	3	121.8 ± 5.5	104.4 ± 4.7	87.0 ± 3.9	69.6 ± 3.2

Didinium density (#/20 mL):
Block 1: 3, 6, 9, 12, 15
Block 2 and 3: 1, 2, 3 (300 μg/L: 4, 5, 6, 7)

Intrinsic Rates of Natural Increase

Experiments to determine rates of increase were performed in 50-mL glass beakers. Initial densities of *Paramecium* (142.59 ± 11.19; $X \pm$ SE) and *Didinium* (11.86 ± 0.98) were determined by counting 20 0.5-mL samples from the stock culture. One mL of the stock culture was then added to 19 mL of growth medium. After 24 h, four 0.5-mL samples were taken and all animals were counted. The intrinsic rate of increase (r) was calculated, using the formula: $r = (\ln N_t - \ln N_0)/t$ where N_t is the final population number/20 mL, N_0 is the initial population number/20mL, and t is the time in days. Four replicates at each copper concentration were performed during each of three blocks over time. A two-way factorial analysis of variance was used to determine if significant differences existed among blocks or copper concentrations.

Isocline Analysis

Experiments to determine the equilibrium point of the predator–prey interaction were also performed in 50-mL glass beakers. Initial densities of *Didinium* were precisely determined by pipetting appropriate numbers of individuals into beakers. Initial densities of *Paramecium* were determined as described above. All initial densities for the isocline analysis are shown in Table 1. An appropriate volume of the stock culture was then used to produce the desired *Paramecium* densities in experimental beakers containing 20.0 ± 0.2 mL culture and growth medium. These cultures were allowed to incubate for 24 h after which time four 0.5-mL samples were taken from each beaker and the numbers of both *Paramecium* and *Didinium* were recorded. Four replicates at each combination of densities were run during each of three blocks over time.

The first block used a wide range of predator and prey densities to identify the region where the equilibrium point existed. The other two blocks used initial predator and prey densities that were concentrated around the equilibrium point. The placement of the prey isocline was determined by performing a regression on the final number of *Paramecium*, at the

TABLE 2. (A) *Paramecium* and (B) *Didinium* densities for 0 μg Cu/L after 24 h incubation. Changes in density were used in the construction of the prey isocline. Initial *Paramecium* densities are given in Table 1. An asterisk indicates an increase in density. All numbers are given as individuals per 20 mL ± SE.

Block	Initial *Didinium*	Density *Paramecium* at 24 h			
A. 1	3	362.5 ± 18.9 (*)	80.7 ± 7.1 (*)	0	0
	6	305.0 ± 26.6 (*)	35.0 ± 12.6	5.0 ± 5.0	2.5 ± 2.5
	9	32.5 ± 26.3	0	0	0
	12	27.5 ± 13.8	2.5 ± 2.5	0	2.5 ± 2.5
	15	2.5 ± 2.5	2.5 ± 2.5	0	0
2	1	115.0 ± 21.0 (*)	85.0 ± 22.5 (*)	37.5 ± 16.0 (*)	12.5 ± 2.5
	2	67.5 ± 14.9 (*)	15.0 ± 2.9	25.0 ± 9.6	5.0 ± 2.9
	3	25.0 ± 13.2	15.0 ± 11.9	0	0
3	1	77.5 ± 40.0 (*)	50.0 ± 10.8 (*)	35.0 ± 15.0 (*)	0
	2	75.0 ± 40.0	2.5 ± 2.5	0	0
	3	85.0 ± 63.8 (*)	2.5 ± 2.5	10.0 ± 10.0	0
B. 1	3	22.5 ± 11.1 (*)	47.5 ± 13.1 (*)	0	0
	6	67.5 ± 9.5 (*)	67.5 ± 18.9 (*)	12.5 ± 9.5 (*)	2.5 ± 2.5
	9	50.0 ± 30.3 (*)	50.0 ± 14.7 (*)	22.5 ± 6.3 (*)	5.0 ± 2.9
	12	65.0 ± 10.4 (*)	47.5 ± 11.1 (*)	35.0 ± 13.2 (*)	25.0 ± 11.9 (*)
	15	145.0 ± 47.9 (*)	102.5 ± 17.0 (*)	17.5 ± 7.5 (*)	10.4 ± 4.1
2	1	10.0 ± 4.0 (*)	2.5 ± 2.5 (*)	0	5.0 ± 5.0 (*)
	2	10.0 ± 7.0 (*)	15.0 ± 2.9 (*)	22.5 ± 2.5 (*)	0
	3	15.0 ± 8.7 (*)	30.0 ± 12.2 (*)	22.5 ± 7.5 (*)	2.5 ± 2.5
3	1	27.5 ± 6.3 (*)	15.0 ± 2.9 (*)	27.5 ± 21.0 (*)	22.5 ± 4.8 (*)
	2	45.0 ± 10.4 (*)	30.0 ± 8.2 (*)	15.0 ± 6.5 (*)	0
	3	47.5 ± 8.5 (*)	22.5 ± 13.1 (*)	12.5 ± 9.5 (*)	2.5 ± 2.5

highest initial *Paramecium* density, versus the initial number of *Didinium*. Using this regression line it was possible to determine the number of *Didinium* required to just maintain the *Paramecium* density. This density combination represents a point on the prey isocline. This method agreed well with the placement of the isocline by eye as done by Maly (1969). The predator isoclines were also fitted to the data by eye by mapping the changes in predator and prey density using vectors whose length and direction represented the changes in magnitude and direction of the population densities over the 24-h period. Each isocline is then drawn as the line of best fit separating increases in density from decreases in density. The intersection of the predator and prey isoclines represents an equilibrium point. Data used in the construction of the control isoclines is presented in Tables 2a and 2b, and Fig. 1 demonstrates the use of these data in the construction of the isoclines.

The following method was used to determine the stability properties of the interactions:

(1) The origin of the graph was transposed to the equilibrium point.

(2) The rates of increase of predator and prey were assumed to be directly proportional to the distances to the left or right

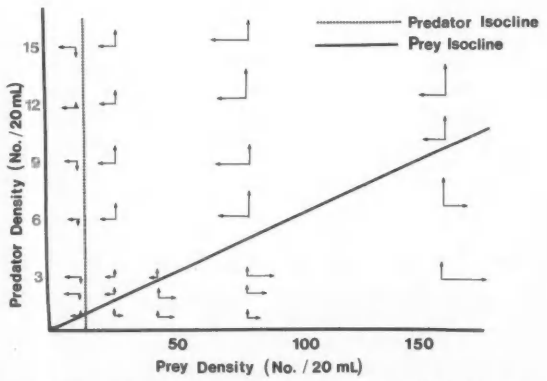

FIG. 1. Vectors representing magnitude and direction of changes in densities of predator (vertical) and prey (horizontal) under control (0 μg/L CU) conditions. Isoclines are drawn as the lines of best fit separating increases in density from decreases in density.

of the predator isocline and above or below the prey isocline, respectively.

(3) Equations were then determined using a linear approximation of the slope of the prey isocline. (The predator isocline was vertical:

[1] $dx/dt = \alpha(kx - y)$

[2] $dy/dt = \beta x$

where α and β are proportionality constants, k is the slope of the isocline, x is the prey density, and y is the predator density.

(4) Solutions for these were written as follows:

[3] $x = Ae^{mt}$

[4] $y = Be^{mt}$

and the common parameter, m, was calculated as:

$$[5] \quad m = \frac{\alpha k}{2} \pm \frac{\sqrt{(\alpha k)^2 - 4\alpha\beta}}{2}$$

(5) The sign of m determines whether the interaction is stable (m is negative) or unstable (m is positive) (Rosenzweig and MacArthur 1963).

Results

Copper Toxicity

The survival abilities of both *Paramecium* and *Didinium* decrease as copper concentration increases (Tables 3a, b). A multiple regression analysis with repeated measures indicated that while there is no significant difference in survival during the first 8 h at different copper concentrations, survival decreases after the 8-h sampling period. There is a significant effect of time, and variation between and within beakers is significant. However, there is a definite trend towards decreasing survivorship with increasing copper concentration.

The copper concentrations used appear to have no effect on the survival of *E. coli* (Table 4). All of the plates contained colonies after the 24-h incubation indicating the presence of viable cells. A chi-square analysis indicated no significant differences ($p > 0.05$) in the number of colonies which are formed at any of the copper concentrations tested, suggesting that *Paramecium* always have a sufficient food supply and that food shortage did not affect the predator–prey interaction.

Rate of Increase

Both *Paramecium* and *Didinium* have significantly higher rates of increase at 30 μg Cu/L when compared with the controls (Tables 5a, b). *Paramecium* show a decline in their rate of increase at higher copper levels although it remains significantly higher than the control rate. *Didinium* rates of increase continue to rise up to a concentration of 180 μg Cu/L but then fall, becoming negative at 300 μg Cu/L.

Isocline Analysis

The equilibrium density of the prey tends to decrease as copper concentration increases (Fig. 2a). At 300 μg Cu/L the predator isocline has shifted to the right and the slope of the prey isocline has increased (Fig. 2b). The equilibrium thus occurs at a much higher density of both predator and prey. This change in equilibrium at 300 μg Cu/L occurs because at this concentration copper is no longer sublethal for *Didinium*.

Using the mathematical analysis as described by Maly (1969) we can demonstrate that the stability of the interaction decreases with increasing copper concentration. The equilibrium point of the interaction occurs at the cartesian coordinates (18,1) under control conditions (Fig. 2a). The values for the constants a and b were determined using differential equations. Substituting the values of these constants, as well as k, the slope of the prey isocline, into equation (5) the value of the common parameter, m, was determined to be:

$$m = 0.416 \pm 1.459i.$$

Since m is positive and the values of x and y will increase with time (equations (3) and (4)), the equilibrium point is unstable.

TABLE 3. Effect of copper on survival of (A) *Paramecium* and (B) *Didinium*. Each number is $N_t/N_0 \pm$ SE.

[Cu] (μg/L) (Nominal)	Time (h)					
	4	8	12	16	20	24
A.						
0	1.4±0.1	1.3±0.1	2.6±0.4	3.7±0.1	5.7±0.2	7.1±0.1
150	1.2±0.1	1.6±0.3	1.7±0.2	3.3±0.4	4.8±0.1	6.4±0.3
300	1.4±0.1	1.3±0.1	2.0±0.1	2.5±0.4	3.6±0.4	5.4±0.1
600	1.4±0.6	1.8±0.4	1.9±0.2	2.2±0.1	2.8±0.7	3.9±0.2
900	1.4±0.7	1.4±0.1	2.3±0.8	1.1±0.9	2.2±1.2	2.7±0.2
1200	1.5±0.4	1.0±0.5	1.2±0.6	1.1±0.2	1.4±1.0	1.3±0.9
1500	1.2±0.1	1.0±0.2	0.5±0.2	0.5±0.1	0.3±0.1	0.0
p[a]	0.984	0.398	0.043	0.003	0.012	0.003
B.						
0	1.1±0.6	2.8±0.8	8.2±1.1	31.3±9.2	26.7±0.2	28.9±1.3
150	1.5±0.4	3.4±1.7	6.4±0.9	20.6±3.2	19.9±2.3	21.2±0.1
300	1.3±0.4	2.3±1.1	1.1±0.6	1.0±0.7	1.0±0.7	1.2±0.5
600[b]	1.0±0.7	1.0±0.7	1.0±0.7	1.0±0.7	1.0±0.7	1.0±0.7
p[a]	0.646	0.280	0.004	0.016	0.008	0.098

[a] p values are for the effects of copper within each time interval.
[b] No animals survived at concentrations exceeding 600 μg/L Cu.

TABLE 4. The number of *E. coli* colonies present on EMB agar after 24 h incubation with various copper concentrations.

[Cu] (μg/L)	Colonies (number)
0	34
30	15
60	33
180	27
300	22

TABLE 5. Intrinsic rates of increase (r) of (A) *Paramecium* and (B) *Didinium* for test copper concentrations. Analysis of covariance and Newmann-Keuls tests were used on log $(X+1)$ (A) or log $(X+3)$ (B) transformed data to test for significance.

[Cu] (μg/L)	$r \pm$ SE	p^a
A.		
0	0.79 ± 0.06	—
30	1.34 ± 0.08	<0.001
60	1.06 ± 0.11	<0.001
180	1.02 ± 0.07	<0.001
300	0.90 ± 0.06	0.05>p>0.025
B.		
0	2.5 ± 0.08	—
30	3.0 ± 0.09	<0.001
60	3.2 ± 0.15	<0.001
180	3.6 ± 0.07	<0.001
300	−0.5 ± 0.21	<0.001

[a]p values are for comparison with control.

A similar analysis was performed to examine changes in the stability of the equilibrium point with the addition of copper. The values of α, β, b, k, and m under the five test conditions are presented in Table 6. There is a trend towards increasing m values with increasing copper concentration. The imaginary component of the m-values represents oscillatory behaviour around the equilibrium point. The period of oscillation is: $2\pi/b$ is the real component of the i term. Since b increases with copper concentration, the period of oscillation decreases until 180 μg Cu/L where the oscillation term is lost (Table 6).

Discussion

The results of the acute toxicity tests indicate that copper is more toxic to *Didinium* than to *Paramecium* (Tables 3a, b). Because of this observation we expected to find that copper would decrease the predator rate of increase or decrease the predators' efficiency at capturing or ingesting prey resulting in changes in the equilibrium densities of both predator and prey as Emlen (1984) demonstrated. We predicted that the equilibrium densities of both predator and prey would increase with the addition of copper at sublethal levels because the predator would not be as efficient at capturing or consuming prey, thus reducing the predation pressure and allowing the prey equilibrium density to increase. An increase in the prey equilibrium density would also mean that more predators would be maintained in the population, resulting in an increase in the predator population density at equilibrium as well. However, the stability properties of the system, which are critical to a knowledge of environmental impact, cannot be described without additional analysis.

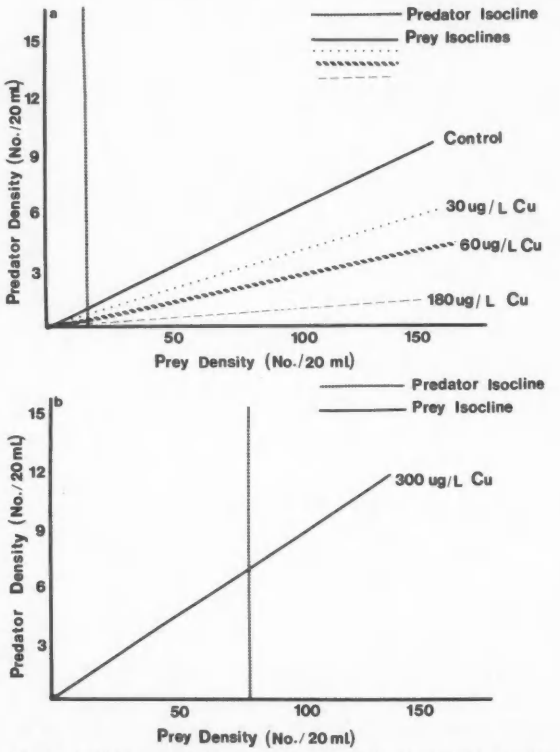

FIG. 2. Isocline analysis for copper concentrations tested. (a) Isocline positions for sublethal copper levels; (b) isoclines for 300 μg Cu/L which is not sublethal for *Didinium*.

TABLE 6. Calculated values (α, β, k, b, and m) for the predator–prey interaction at test copper concentrations.

[Cu] (μg/L)	α	β	k	b	m
0	14.85	0.155	0.056	1.459	0.416 ± 1.459i
30	26.00	0.455	0.046	3.349	0.598 ± 3.349i
60	29.90	15.99	0.031	23.37	0.530 ± 23.37i
180	98.40	−0.008	0.007	—	1.296
300	10.60	−0.064	0.085	—	1.390

The changes that were predicted based on the acute lethality tests were not observed with the isocline analysis, except at the highest copper concentration tested (Fig. 2b). Instead, the equilibrium density of *Didinium* tended to decrease and there was no apparent change in the equilibrium density of *Paramecium* (Fig. 2a). This response occurred because of the toxicants' effect on the intrinsic rates of increase for both species. The addition of copper resulted in higher rates of increase (Tables 5a, b). This phenomenon, called hormesis (Southham and Ehrlich 1943) has been observed in *Daphnia magna* exposed to toxicant stress (Dave 1984; Bodar et al. 1988) and in *Didinium* subjected to nutritional stress (Hewett 1980).

Hormesis is more pronounced in *Didinium* than in *Paramecium* and results in a destabilization of the predator–prey interaction at low copper levels (Table 6). Destabilization continues with increasing copper concentration but the mode of destabilization shifts from one caused by differential hormesis to one

caused by a decrease in predator efficiency as predicted from the acute lethality tests.

Attention has only recently begun to focus on the effects of toxicants at the community and ecosystem levels (Lawrence and Holoka 1979; NRC 1981; Cairns 1983; Kooijman and Metz 1984; McDowell Capuzzo et al. 1988; Pratt et al. 1988). These studies demonstrate that multispecies tests should be used to predict the impact of contaminants on higher levels of biological organization. Alterations in the community composition, species diversity, and densitites cannot be predicted accurately from single species tests (O'Neill et al. 1982). However, single species acute and chronic toxicity tests have provided the majority of the information currently available on the effects of toxicants and will undoubtedly continue to serve a role in obtaining information on toxicant impact on the ecosystem.

The results of this study demonstrate the need for a knowledge of general ecological parameters if attempts to predict the impact of toxicants at the ecosystem level are to be made. The relevant ecological parameters for a predator–prey system would include:

(1) changes in the abilities of both organisms to withstand the toxicant,

(2) changes in predator efficiency at consuming prey, relative to the prey rate of increase and,

(3) changes in rates of increase as toxicant levels increase. The examination of the effects of toxicants on a predator–prey interaction integrates a number of factors such as lethality, reproduction, and predator–prey behavior. All of these factors must be considered if the overall effect of the toxicant is to be observed and community and ecosystem level effects are to be more precisely predicted and understood.

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Weighted Multicollinearity in Logistic Regression: Diagnostics and Biased Estimation Techniques with an Example from Lake Acidification

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Marx, B. D., and E. P. Smith. 1990. Weighted multicollinearity in logistic regression: diagnostics and biased estimation techniques with an example from lake acidification. Can. J. Fish. Aquat. Sci. 47: 1128–1135.

An historical data set from the Adirondack region of New York is revisited to study the relationship between water chemistry variables associated with acid precipitation and the presence/absence of brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*). For the trout species data sets, water chemistry variables associated with acid precipitation, for example pH and alkalinity, are highly correlated. Regression models to assess their effects on the probability of the presence of fish species are therefore affected by multicollinearity. Because the appropriate regressions are logistic, correction techniques based on least squares do not work. Maximum likelihood parameter estimation is highly unstable for the trout presence/absence data. Developments in weighted multicollinearity diagnostics are used to evaluate maximum likelihood logistic regression parameter estimates. Further, an application of biased parameter estimation is presented as an option to the traditional maximum likelihood logistic regression. Biased estimation methods, like ridge, principal component, or Stein estimation can substantially reduce the variance of the parameter estimates and prediction variance for certain future observations. In many cases, only a slight modification to the converged maximum likelihood estimator is necessary.

Un ensemble de données chronologiques pour la région des Adirondacks de l'État de New York est réexaminé pour étudier la relation entre les variables de la chimie de l'eau associées aux pluies acides et la présence/l'absence de l'omble de fontaine (*Salvelinus fontinalis*) et du touladi (*Salvelinus namaycush*). Dans le cas des ensembles de données sur les espèces de truite, les paramètres de la chimie de l'eau associés aux précipitations acides, comme le pH et l'alcalinité, sont fortement corrélés. La multicolinéarité influe donc sur les modèles de régression pour l'évaluation des effets sur la probabilité de la présence d'espèces de poisson. Les techniques de correction basées sur la méthode des moindres carrés ne fonctionnent pas car les régressions appropriées sont logistiques. L'estimation du paramètre de maximum de vraisemblance est très instable dans le cas des données sur la présence/l'absence de truite. Les diagnostics de multicolinéarité pondérée sont développés pour évaluer les estimations du paramètre de maximum de vraisemblance de la régression logistique. De plus, une application de l'estimation d'un paramètre biaisé est présentée à titre de solution de rechange à la régression logistique conventionnelle du maximum de vraisemblance. Les méthodes d'estimation biaisée, comme l'estimation par crête, par composante principale ou de Stein, peuvent énormément réduire la variance des estimations du paramètre et des prédictions dans le cas de certaines observations futures. Dans de nombreux cas, il suffit d'apporter une légère modification à l'estimateur convergent du maximum de vraisemblance.

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There has recently been international concern and effort in estimating the probability of presence/absence of various fish species from models involving water chemistry variables responsible for lake acidification (Christensen et al. 1988; Reckhow et al. 1987; Baker 1984; Magnuson et al. 1984; Howells 1983; Haines 1981). Estimating and interpretating how the probability of presence depends on attributes of water chemistry which can have a great impact on efforts to prevent the loss of ecologically and economically important species.

The analysis of data on the presence or absence of fish species is different from ordinary regression analysis for two reasons. First, the response variable (presence or absense) is a binary variable which is one of two values and hence is not normal. The approach typically used to model this type of data is logistic regression. In logistic regression, the probability of presence is modelled as a logistic response function which depends on the water chemistry variables and other explanatory variables. Estimation of the parameters for the model is done via an extension of the least squares method called iterative reweighted least squares.

The second difference involved multicollinearities. The water chemistry variables associated with acid precipitation, such as pH and alkalinity, are often highly correlated. pH measures the concentration of hydrogen ion in the water while alkalinity is a measure of acid neutralizing capacity of the water. Alkalinity is dependent on pH but also reflects buffering capacity of the

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water system. Thus there is a strong but not perfect relationship between these variables. Linear or near linear dependencies between the explanatory variables are called multicollinearities and can adversely affect the results of regression analysis. Management strategies which attempt to control one physical–chemical variable based on a regression analysis may be misled as to the effect of controlling that variable. Either the joint effets of variables need to be considered or the effect of a single variable needs to be better estimated in the presence of other variables. While there is much information on the effects, diagnosis, and adjustment of multicollinearity in multiple regression analysis, little work has been done for the logistic regression problem.

Recent advances in the statistical literature (Schaefer 1986; Marx and Smith 1989) offer improved ways to analyze data resulting from studies concerned with the presence/absence of fish species due to changes in water chemistry. It is the objective of this paper to describe weighted multicollinearity diagnostics for logistic regression. These diagnostics can assist in identifying variables to be considered for potential deletion from the model. Further, biased estimation techniques are presented as an option to traditional maximum likelihood logistic regression. Ridge logistic, principal component logistic, and Stein logistic models will be formulated and interpreted for brook trout (*Salvelinus fontinalis*) and lake trout (*Salvelinus namaycush*) from the Adirondack region of New York. These biased estimation techniques can be particularly useful when theoretical models of interest have severe, or even moderate, multicollinearity problems. The techniques are applied to the data of Reckhow et al. (1987). Reckhow et al. described the problems of multicollinearity and presented alternatives to ordinary logistic regression. Their methods however, were based on the techniques used for multiple regression analysis and did not take into account the special structure of the data. The methods presented here lead to improved results.

Brief Overview of Logistic Regression

Hosmer and Lemeshow (1989) provides an excellent overview of logistic regression. Logistic regression is commonly used to model the probability of a dichotomous outcome when given explanatory variables of interest. Consider response data that is binary in nature, such as presence/absence of trout, and suppose there are N observations and p explanatory variables. Define the $N \times (p + 1)$ matrix, $X = (1\ x_1 \ldots x_p)$. The x_j are continuous explanatory variables. Denote x_i', of dimension $1 \times (p + 1)$, as a row vector of X. Let β be the $(p + 1) \times 1$ vector of unknown regression parameters. The response variable is the probability of presence which depends on the explanatory variables. This probability that a fish species is found in lake i is defined as the conditional probability, $\pi_i = P(Y_i = 1|x_i')$. The response probability is then modelled as a sigmoidal curve which depends on the explanatory variables measured in the lake. The logit or log odds ratio model uses the cumulative logistic density which ensures the predicted probability is between 0 and 1. Other cumulative densities can be used to form an appropriate model, but are not considered in this paper. Given independent Bernoulli response data $Y_i (i = 1,2, \ldots, N)$, let Y be the $N \times 1$ binary response vector consisting of ones and zeros. The logistic regression model can be formulated,

$$(1)\quad \text{logit}(\pi_i) = \ln\{\pi_i(1-\pi_i)^{-1}\} = x_i'\beta$$

$$(2)\quad \text{or} \qquad \pi_i = \{1 + \exp(-x_i'\beta)\}^{-1}.$$

Because the distribution of Y is neither reasonably continuous nor symmetric, ordinary least squares is not an appropriate estimation technique and iterative maximum likelihood is commonly used to estimate the unknown regression parameters. The iterative maximum likelihood scheme for logistic regression can be expressed as

$$(3)\quad \hat{\beta}_t = \hat{\beta}_{t-1} + (X'\hat{V}_{t-1}X)^{-1}X'(y - \hat{\pi}_{t-1}),$$

where t denotes the iteration step, $\hat{V} = \text{diag}\{\hat{\pi}_i(1 - \hat{\pi}_i)\}$, and $\hat{\pi}$ is the vector of predicted probabilities. Note that the procedure is a weighted procedure, and that instead of using $X'X$, one uses $X'VX$. The diagonal matrix V contains variances of the estimated Y values. The matrix $\Phi = X'VX$ is called the information matrix. Denote $\hat{\Phi} = X'\hat{V}X$ as estimated information. An ill-conditioned information matrix can result in undesirable asymptotic properties of the logistic regression, such as large variances associated with parameter estimates and certain prediction regions. Analogous to what is done in multile regression, the effects of multicollinearity can be assessed by decomposing the information matrix into orthogonal components. Let G be the orthogonal matrix such that $G'\hat{\Phi}G = \Lambda$, where $\Lambda = \text{diag}\{\lambda_j\}$ and λ_j are the eigenvalues of $\hat{\Phi}$. Define $Z = XG$ and z_u' as a row of Z. As sample size gets large, the variance of the coefficients, $\text{Var}(\beta) \cong \hat{\Phi}^{-1}$. If $\hat{\Phi}$ has a small λ_j, then some examples of undesirable properties of maximum likelihood estimation include:

(a) extreme sensitivity of parameter estimates to small perturbations in explanatory variables.
(b) The sum of the variances of the coefficients tends to infinity.
(c) For certain future observation data vectors x_u', the prediction variance is approximately
$\text{Var}(\hat{\pi}_u) \cong \{\pi_u(1 - \pi_u)\}^2 \Sigma_{j=0}^{p} z_{u,j}^2 \lambda_j^{-1} \to \infty$.
(d) The test:
$H_0 : \beta = \beta_c$
$H_1 : \beta = \beta_f$,
may have low power, where c and f denote the current and full model, respectively (c is typically a subset of f).

The problems above resemble those of least squares multicollinearity. Multicollinearity results when one explanatory variable can be nearly expressed as linear combination of the remaining explanatory variables. Redundancies in the regressors yields multicollinearity. With multicollinearity in a standard multiple regression, the least squares estimation technique is not exposed to a regressor variable data structure that it truly needs to produce clear estimates of rate of change on the response variable (Myers 1990). Traditionally, with least squares estimation in standard multiple regression, spectral decomposition of the correlation matrix of explanatory variables has been used as a diagnostic tool to determine the ill-effects of multicollinearity on parameter estimation and prediction. Researchers have been unjustifiably utilizing these least squares multicollinearity diagnostics for models not based on least squares, logistic regression is one example. Many of the assumptions of least squares linear regression are not met. Certainly a response which is a 0 or a 1 fails to meet normality of error terms. Furthermore, Bernoulli variances are heteroscedastic.Theoretical investigation (Marx and Smith 1989) suggested that logistic regression diagnostics should be oriented toward the spectral decomposition of $X'\hat{V}X$, where $\hat{V} = \text{diag}\{\hat{\pi}_i(1 - \hat{\pi}_i)\}$ is a diagonal matrix of Bernoulli variances. Multicollinearity diagnostics can be formulated among the weighted explanatory variables, i.e. the columns of $\hat{S} = \hat{V}^{1/2}X$.

These diagnostics often yield more pertinent information for selection of a candidate model, in that they can be used as a variable selection tool.

Weighted Multicollinearity Diagnostics for Logistic Regression

A matrix $\hat{\Phi}$ is ill-conditioned if it has nearly linearly dependent columns of $\hat{S} = \hat{V}^{1/2}X$. For X with full rank, notice that the strength of the linear dependence among the columns of X does not directly affect the condition of $\hat{\Phi}$ unless the diagonal matrix $\hat{V}^{1/2} \cong kI$. In such a special case, not only are the binary Y_i independent, but they further have nearly homogeneous variance. Diagnostics should be developed accordingly. In developing suitable diagnostics, scaling of the information matrix is preferred in order to have a standard for comparison (Belsley et al. 1980). A natural scaling method which gives the columns of $\hat{S}$ unit length is given in equation (4). Let

$$(4) \quad \hat{S}^*(i,j) = (\hat{S}_{ij} - \bar{S}_j) \left[\sum_{i=1}^{N} (\hat{S}_{ij} - \bar{S}_j)^2 \right]^{-1/2}.$$

Define the weighted correlation matrix $\hat{\Phi}^* = \hat{S}^{*\prime}\hat{S}^*$. *The* (i,j)th entry of $\hat{\Phi}^*$ yields the correlation between the ith and jth column of $\hat{S}$.

In assessing the degree of ill-conditioning of a general matrix B, Hartree (1952) pointed out the importance of a relative measure of ill-conditioning called a condition number. Belsley et al. (1980) argued that if a small eigenvalue is used as a multicollinearity diagnostic, then there is a natural tendency to compare small with the wrong standard, namely zero. Hence, the ratios of functions of eigenvalues, rather than the eigenvalues alone, have been useful diagnostics. Condition numbers are defined for the purpose of this paper as the ratio of the largest to the jth smallest eigenvalue of $\hat{\Phi}^*$,

$$(5) \quad \Psi_j = (\lambda^*_{\max}/\lambda^*_j)^{1/2},$$

where the λ^*_j were the ordered eigenvalues of $\hat{\Phi}^*$. Large values of Ψ_j (≥ 30) indicate ill-conditioning.

Weighted Variance Inflation

For least squares estimation, variance inflation factors (VIFs) indicate the inflation of variance above the ideal, i.e. the correlation matrix of explanatory variables being the identity matrix. Constructing such a diagnostic for logistic regression is a bit more complicated. For one, the covariance matrix for $\hat{\beta}$ is not a scalar multiple of $X'X$. Perhaps the most obvious construction of weighted variance inflation factors (WVIFs) for logistic regression is to consider the diagonal elements of $\hat{\Phi}^{*-1}$. Under ideal conditions, i.e. when the columns of $\hat{S}$ are orthogonal, $\hat{\Phi}^* = \hat{\Phi}^{*-1} = I$ and all $\Psi_j = 1$. Marx and Smith (1989) quantified a measure of inflation of asymptotic variance due to the nonorthogonality among the columns of $\hat{S}$. Define

$$(6) \quad \text{WVIF}_j = j\text{th diagonal element of } \hat{\Phi}^{*-1},$$

as a weighted variance inflation factor. Observe that there is WVIF associated with the intercept since the centering and scaling of $\hat{S}$ does not involve a constant column of ones, but rather $\hat{V}^{1/2}1$. Notice for the special case of $V = I$ and regression through the origin, WVIF reduces to a least squares VIF. A WVIF gives the inflation of variance above the standard of one under ideal conditions.

Weighted Variance Proportion

Once the WVIFs are calculated, it also can be informative to determine to what extent the proportion of variance of each coefficient is attributed to each near dependency in the information matrix. Weighted variance proportion decomposition requires the spectral decomposition of $\hat{\Phi}^*$. Let M be the orthogonal matrix such that $M'\hat{\Phi}^*M = \Lambda^*$, where $\Lambda^* = \text{diag}\ \{\lambda^*_j$ and λ^*_j are the eigenvalues of $\hat{\Phi}^*$. Using the asymptotic correlation form covariance matrix for the converged maximum likelihood parameter estimates, define

$$(7) \quad C_{jj} = \sum_{u=0}^{p} m^2_{ju}/\lambda^*_u.$$

A small eigenvalue has influence, to some degree, on all variances. The weighted proportion of variance for the jth estimated coefficient, attributed to uth eigenvalue of the sum in equation (7), can be expressed as

$$(8) \quad \rho_{uj} = \frac{m^2_{ju}/\lambda^*_u}{C_{jj}}.$$

Weighted variance proportion decomposition

Ordered Eivengalue	Proportion of Var $(\hat{\beta}_0)$	Var $(\hat{\beta}_1)$	...	Var $(\hat{\beta}_p)$
λ_0	ρ_{00}	ρ_{01}	...	ρ_{0p}
λ_1	ρ_{10}	ρ_{11}	...	ρ_{1p}
.	.	.		.
.	.	.		.
.	.	.		.
λ_p	ρ_{p0}	ρ_{p1}	...	ρ_{pp}

A matrix of proportions can be constructed. Hence, a small eigenvalue (relative to the maximum eigenvalue) responsible for at least two large proportions suggests that weighted multicollinearity is damaging desirable properties of the logistic regression. Action should be taken accordingly. If a large WVIF is present, then the researcher should consider deletion of one of the variables responsible for a large ρ_{uj} (associated with a small eigenvalue) or an alternative estimation technique, for example, a biased logistic estimation technique (Schaefer 1986). If specific theoretical models are not of interest, then perhaps wary variable deletion based on the above diagnostics can satisfy the researcher's needs for a predictive model. However, in instances where a coefficient estimate is of primary concern, variable deletion may not be reasonable.

To illustrate the effects of the ill-conditioned information matrix, we consider the models in Table 1 which were presented in Reckhow et al. (1987). Using brook trout presence/absence data from Adirondack lakes, Table 1 provides a model uses explanatory variables: lake pH, calcium content (log transformed), and the interaction between these two variables. Table 1 also provides a model for presence/absence of lake trout. Explanatory variables used are lake pH, alkalinity (log transformed), and the interaction term. Reckhow et al. (1987) provide plots justifying the importance and interpretation of the interaction terms for both species of trout. Table 1 provides a deviance goodness-of-fit measure. The deviance measures how well the model fits the data and is defined as $D = \sum_{i=1}^{N} d_i^2$, where $d_i^2 = -2\ln(1 - \hat{\pi}_i)$ for $y = 0$ and $d_i^2 = -2\ln(\hat{\pi}_i)$ for $y = 1$.

TABLE 1. Maximum likelihood estimates, standard errors, and deviance for Reckhow et al. (1987) trout data.

	$\hat{\beta}$	SE($\hat{\beta}$)	D
Brook trout ($N=46$)			
Intercept	−49.8460	26.4290	43.2936
pH	9.1906	3.9094	
In(Ca)	8.0101	5.9051	
pH*In(Ca)	−1.4885	0.8249	
Lake trout ($N=32$)			
Intercept	−233.4000	127.6000	34.4160
pH	32.3834	16.6454	
In(Alk)[a]	45.6460	26.0854	
pH*In(Alk)	−6.3188	3.4195	

[a]Alkalinity shifted + 150 to create all positive values.

TABLE 2. Logistic weighted muticollinearity diagnostics for brook trout data ($N=46$).

Correlation matrix of weighted regressors

	Intercept	pH	In(Ca)	pH*In(Ca)
Intercept	1.0000	0.8084	0.8794	0.6326
pH	0.8084	1.0000	0.9615	0.9554
In(Ca)	0.8794	0.9615	1.0000	0.9138
pH*In(Ca)	0.6326	0.9554	0.9138	1.0000

Weighted variance inflation factors

Intercept	235.5
pH	292.8
In(Ca)	384.0
pH*In(Ca)	564.5

Variance proportion decomposition

E-value	Ψ_j	Intercept	pH	In(Ca)	pH*In(Ca)
3.5846	1.0000	2.5E-04	2.6E-04	2.0E-04	1.2E-04
0.3857	3.0484	0.0067	3.0E-04	1.6E-05	0.0016
0.0290	11.1146	0.0018	0.0593	0.0419	9.9E-04
6.9E-04	71.8325	0.9912	0.9401	0.9578	0.9973

Asymptotic arguments suggest that D has a limiting (with sample size) χ^2_{N-p-1} distribution.

Although the fit of these above models seems reasonable, there are some disturbing problems. The magnitude and standard errors of the coefficients for these models are inflated relative to models containing only one variable. For example, when the presence of lake trout is modelled in terms of pH alone, the estimated coefficient is 0.923 (SE = 0.457), while the three variable model results in a coefficient of 32.38 (SE = 16.65). Further evidence of problems are given by the weighted multicollinearity diagnostics for the brook trout data (Table 2) and the lake trout data (Table 3). Notice the large positive correlations among the weighted regressors yielding extremely large weighted variance inflation factors, especially in the lake trout data. For both data sets, we find a large condition number (≥30) with the smallest eigenvalue of the information matrix. The decomposition matrix has a last row of values all nearly one indicating a redundancy among the explanatory variables. Certainly an option is to drop the interaction term from both models. Even though this may be a reasonable approach, the coefficient of interaction may be of interest. As an option to variable deletion, we consider a variety of biased estimation techniques.

TABLE 3. Logistic weighted muticollinearity diagnostics for lake trout data ($N=32$).

Correlation matrix of weighted regressors

	Intercept	pH	In(Ca)	pH*In(Alk)
Intercept	1.0000	0.8441	0.9423	0.7406
pH	0.8441	1.0000	0.9525	0.9781
In(Alk)	0.9423	0.9525	1.0000	0.9125
pH*In(Alk)	0.7406	0.9781	0.9125	1.0000

Weighted variance inflation factors

Intercept	3180.3
pH	3972.7
In(Ca)	4544.9
pH*In(ca)	6580.2

Variance proportion decomposition

E-value	Ψ_j	Intercept	pH	In(Alk	pH*In(Ca)
3.6885	1.0000	1.9E-05	1.8E-05	1.6E-05	1.0E-05
0.2920	3.5541	5.8E-04	7.0E-05	2.1E-05	1.8E-04
0.0194	13.7845	9.4E-04	0.00569	0.00518	3.6E-04
5.5E-05	259.3000	0.99850	0.99420	0.99480	0.9994

Biased Logistic Regression Estimators

Using Taylor series arguments, it can be shown that the maximum likelihood parameter estimates are asymptotically unbiased. In making certain adjustments to maximum likelihood, asymptotically biased parameter estimates can be constructed. Ridge, principal component, and Stein asymptotically biased estimators are presented in this paper. There is much controversy regarding centering and scaling of explanatory variables (i.e. adjusting each variable to mean zero and unit length). In presenting ridge and principal component estimators below, centering and scaling is done. This is consistent with Myers (1990) and Schaefer (1979, 1986). The notation becomes a bit more complicated. However, there is a connection between the centered and scaled estimated coefficients and the ones in the natural units. Let $X_p=(x_1 \ldots x_p)$. Define the (i,j)th element of X_p^*

$$(9)\quad X_p^*(i,j)=q_j^{-1}(X_{ij}-\bar{X}_j),$$

where $q_j=\{\Sigma_{i=1}^N (X_{ij}-\bar{X}_j)^2\}^{1/2}$. Notice that $X_p^{*\prime}X_p^*$ is the $p\times p$ correlation matrix for the explanatory variables. Augment the matrix X_p^* with the constant column of ones and denote $X^*=(1\ X_p^*)$.

In using X^* as the data matrix, let $\tilde{\beta}$ be the converged maximum likelihood estimate for unknown β^* and $\tilde{V}$ be the corresponding estimated diagonal matrix of Bernoulli variances. Denote $\tilde{\Phi}=X^{*\prime}\tilde{V}X^*$. F is the matrix of eigenvectors and the γ_j are the corresponding of eigenvalues of $\tilde{\Phi}$.

Certainly similar derivations for biased estimators can be made for uncentered and unscaled explanatory variables. In addition, if by design the explanatory variables are measured in the same units, then further standardization may not be needed. It is not this paper's objective to discuss alternate standardizations.

Ridge Logistic Regression Estimators

Schaefer (1979, 1986) suggested

$$(10)\quad \tilde{\beta}_R(k)=(X^{*\prime}\tilde{V}X^*+kI)^{-1}X^{*\prime}\tilde{V}X^*\tilde{\beta}$$
$$=\tilde{\Phi}_k^{-1}\tilde{\Phi}\tilde{\beta}$$

where $k>0$ is called the shrinkage or ridge parameter. Note that this is a simple adjustment to the maximum likelihood estimator. For $k=0$, $\tilde{\beta}_R$ is a maximum likelihood estimator of β^*. Upon choice of k, uncentering and unscaling the estimated coefficients to the natural units can be done. Define

$$(11)\quad \hat{\beta}_{R,j}=q_j^{-1}\tilde{\beta}_{R,j} \text{ for } j=1, 2, \ldots p$$

$$(12)\quad \text{and } \hat{\beta}_{R,0}=\tilde{\beta}_{R,0} - \sum_{j=1}^{p} q_j^{-1}\bar{X}_j\tilde{\beta}_{R,j}.$$

The asymptotic covariance matrix for $\tilde{\beta}(k)$ is $\tilde{\Phi}_k^{-1}\tilde{\Phi}\tilde{\Phi}_k^{-1}$. The asymptotic standard errors associated with the uncentered and unscaled ridge estimates can be expressed as

$$(13)\quad \text{SE}(\hat{\beta}_{R,j})=q_j^{-1}\text{SE}(\tilde{\beta}_{R,j}) \text{ for } j=1, 2, \ldots p$$

$$(14)\quad \text{SE}(\hat{\beta}_{R,0})=\{\text{Var}(\tilde{\beta}_{R,0}) + \sum_{j=1}^{p} (q_j^{-1}\bar{X}_j)^2\text{Var}(\tilde{\beta}_{R,j})$$

$$+2\sum_{i<j}\sum_{j\neq 0} q_j^{-1}q_j^{-1}\bar{X}_i\bar{X}_j\text{Cov}(\tilde{\beta}_{R,i},\tilde{\beta}_{R,j})$$

$$-2\sum_{j=1}^{p} q_j^{-1}\bar{X}_j\text{Cov}(\tilde{\beta}_{R,0},\tilde{\beta}_{R,j})\}^{1/2}.$$

The choice of k is subjective, however, Schaefer recommended a harmonic mean method

$$(15)\quad k=(p+1)/(\tilde{\beta}'\,\tilde{\beta}),$$

which is quite conservative (relatively small) under extreme ill-conditioning of $\tilde{\Phi}$. Marx (1988) has also suggested a ridge trace approach (ridge coefficient estimates versus k) and other approaches for the generalized linear model. Only the ridge trace are presented in the trout examples.

One-Step Principal Component Logistic Regression Estimators

Schaefer (1986) developed a principal component estimator for logistic regression of the form

$$(16)\quad \tilde{\beta}_{pc}^{s}=(X^{*\prime}\tilde{V}\,X^*)^+\,(X^{*\prime}\tilde{V}\,X^*)\,\tilde{\beta}$$
$$=\tilde{\Phi}^+\,\tilde{\Phi}\,\tilde{\beta},$$

where

$$\tilde{\Phi}^+=\sum_{j=0}^{p-r}\gamma_j^{-1}f_jf_j'.$$

Note that $r=p+1-s$ is the number of components deleted. The γ_j are the eigenvalues of $\tilde{\Phi}$ (usually in descending order) and f_j are the corresponding eigenvectors. A conversion of $\tilde{\beta}_{pc}^{s}$ to $\hat{\beta}_{pc}^{s}$, in the natural units, can be made using equations similar to equations (11)–(14).

Iterative Principal Component Logistic Regression Estimators

Marx and Smith (1990) developed an alternate iterative principal component estimator which behaves very closely to Schaefer's one step adjustment to maximum likelihood above in equation (16) in the framework of logistic regression. The iterative method considers fitting the model to the principal components, i.e.

$$(17)\quad \text{logit}(\pi^*)=Z^*\,\alpha^*,$$

TABLE 4. Ridge trace parameter estimates, deviance, and associated standard errors as a function of shrinkage for brook trout data ($N=46$).

k	$\hat{\beta}_{R,0}$	$\hat{\beta}_{R,1}$	$\hat{\beta}_{R,2}$	$\hat{\beta}_{R,3}$	D
0.0000	−49.846	9.19056	8.0101	−1.4885	43.2936
0.0005	−18.787	4.59572	1.3115	−0.5099	45.0160
0.0010	−11.965	3.53317	−0.1081	−0.2927	46.0002
0.0015	−9.019	3.03896	−0.6867	−0.1973	46.5424
0.0020	−7.402	2.74195	−0.9791	−0.1439	46.8974
0.0025	−6.395	2.53725	−1.1418	−0.1098	47.1588
0.0030	−5.719	2.38370	−1.2356	−0.0861	47.3674
0.0035	−5.239	2.26180	−1.2893	−0.0689	47.5436
0.0040	−4.887	2.16107	−1.3178	−0.0557	47.6985
0.0045	−4.620	2.07535	−1.3300	−0.0453	47.8387
0.0050	−4.414	2.00079	−1.3311	−0.0370	47.9682

k	$\text{SE}(\hat{\beta}_{R,0})$	$(\hat{\beta}_{R,1})$	$\text{SE}(\hat{\beta}_{R,2})$	$\text{SE}(\hat{\beta}_{R,3})$
0.0000	26.4290	3.9094	5.9051	0.8249
0.0005	9.9936	1.6018	2.5119	0.3007
0.0010	6.5147	1.1474	1.8643	0.1843
0.0015	5.0815	0.9668	1.6094	0.1332
0.0020	4.3309	0.8706	1.4720	0.1046
0.0025	3.8820	0.8093	1.3825	0.0864
0.0030	3.5888	0.7653	1.3165	0.0738
0.0035	3.3848	0.7310	1.2639	0.0645
0.0040	3.2347	0.7028	1.2196	0.0575
0.0045	3.1204	0.6787	1.1810	0.0520
0.0050	3.0304	0.6574	1.1465	0.0476

where $Z^*=X^*F$ and $\alpha^*=F'\beta^*$. The iterative scheme is employed in a specified subset α_s^* and $\alpha_r^*=0$. The iterative equation becomes

$$(18)\quad \tilde{\alpha}_{s,t}^{pc} = \tilde{\alpha}_{s,t-1}^{pc}+\Gamma_{s,t-1}^{-1}\,Z^{*\prime}(y-\tilde{\pi}^{pc}),$$

where t denotes the iterative step until convergence and Γ_s is the diagonal matrix of subset eigenvalues of Φ, which are not deleted. Upon convergence of $\tilde{\alpha}_s^{pc}$, an estimate of β^* is given by

$$(19)\quad \tilde{\beta}_s^{pc}=F\,\tilde{\alpha}_s^{pc}.$$

Again, a conversion can be made to the natural units, using an approach similar to that given in equations (11)–(12), to yield $\hat{\beta}_s^{pc}$. Asymptotically, the standard errors for the one-step and the iterative principal component techniques are the same. Noting that the estimated asymptotic covariance matrix for $\hat{\beta}_s^{pc}$ and $\tilde{\beta}_{pc}^{s}$ is $F_s\Gamma_s^{-1}F_s'$, an equation similar to equations (13)–(14) can be used to obtain the uncentered and unscaled standard errors.

Stein Logistic Regression Estimators

Schaefer (1986) suggested an extension of the Stein (1960) estimator for logistic regression. Consider shrinking the maximum likelihood estimate as follows

$$(20)\quad \hat{\beta}_s=c\,\hat{\beta},$$

where $0<c<1$. The motivation of Stein estimation is to shrink both the estimated parameter vector, and associated standard errors, by a simple scaling technique. One choice of c, which minimizes the $E(L_1^2)=(c\hat{\beta}-\beta)'(c\hat{\beta}-\beta)$ criterion (with respect to c), is

$$(21)\quad c=(\hat{\beta}'\hat{\beta})/\{\hat{\beta}'\hat{\beta}+\text{trace}(\hat{\Phi}^{-1})\}.$$

The standard error of a Stein estimator is c times the standard error of the maximum likelihood estimator.

TABLE 5. Ridge trace parameter estimates, deviance, and associated standard errors as a function of shrinkage for lake trout data ($N=32$).

k	$\hat{\beta}_{R,0}$	$\hat{\beta}_{R,1}$	$\hat{\beta}_{R,2}$	$\hat{\beta}_{R,3}$	D
0.00000	−233.35	32.3834	45.6460	−6.3188	34.4160
0.00025	−27.74	5.5119	3.7373	−0.7934	37.5601
0.00050	−13.16	3.5814	0.7797	−0.3992	38.0683
0.00075	−7.89	2.8670	−0.2797	−0.2552	38.2677
0.00100	−5.20	2.4906	−0.8126	−0.1807	38.3757
0.00125	−3.59	2.2557	−1.1258	−0.1352	38.4447
0.00150	−2.53	2.0933	−1.3268	−0.1046	38.4936
0.00175	−1.79	1.9732	−1.4629	−0.0826	38.5308
0.00200	−1.26	1.8799	−1.5583	−0.0661	38.5606
0.00225	−0.86	1.8046	−1.6265	−0.0532	38.5855
0.00250	−0.55	1.7422	−1.6757	−0.0430	38.6070

k	SE($\hat{\beta}_{R,0}$)	($\hat{\beta}_{R,1}$)	SE($\hat{\beta}_{R,2}$)	SE($\hat{\beta}_{R,3}$)
0.00000	127.6000	16.6454	26.0852	3.4195
0.00025	18.8117	2.3948	4.3011	0.4566
0.00050	12.1473	1.4899	3.1345	0.2459
0.00075	10.1183	1.2046	2.8026	0.1693
0.00100	9.2191	1.0753	2.6537	0.1300
0.00125	8.7276	1.0036	2.5669	0.1062
0.00150	8.4176	0.9581	2.5069	0.0903
0.00175	8.2006	0.9261	2.4604	0.0791
0.00200	8.0361	0.9019	2.4216	0.0707
0.00225	7.9039	0.8825	2.3876	0.0643
0.00250	7.7927	0.8662	2.3568	0.0592

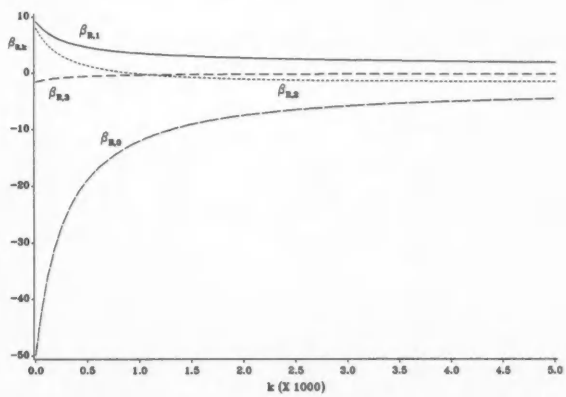

FIG. 1. Ridge trace plot for brook trout estimated regression coefficients as a function of shrinkage.

Application of Methodologies

The above methodologies were applied to the lake chemistry data sets for brook trout and lake trout. Tables 4 and 5 give ridge estimates of the parameters for the two models for different values of the ridge parameter (k). Also, standard errors are provided. Even with small k (0.001), the ridge method is effective in reducing the magnitude of the coefficients and their standard errors, but the sign of the coefficient for the second explanatory varaible, $\hat{\beta}_{R,2}$, differs from the univariate model which only includes the second explanatory variable. Ridge trace plots are given in Fig. 1 and 2 and demonstrate the instability of maximum likelihood estimates. These plots indicate rapid change in the coefficients until k is 0.001. Values near this would represent reasonable values for the ridge coefficient. The trout examples coefficients have been transformed to their

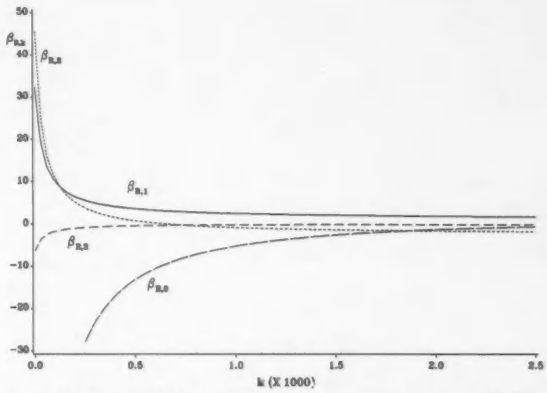

FIG. 2. Ridge trace plot for lake trout estimated regression coefficients as a function of shrinkage.

TABLE 6. Iterative principal component and Stein estimates and associated asymptotic standard errors for brook trout data ($N=46$).

	pc(-1)	pc(-2)	Stein($c=0.78$)
$\hat{\beta}_0$	−0.5382	−5.4530	−38.8111
$\hat{\beta}_1$	2.2315	0.2920	7.1560
$\hat{\beta}_2$	−2.9793	0.5514	6.2369
$\hat{\beta}_3$	0.0528	0.0380	−1.1590
SE($\hat{\beta}_0$)	2.9551	2.3125	20.5781
SE($\hat{\beta}_1$)	0.8189	0.1207	3.0440
SE($\hat{\beta}_2$)	1.4470	0.2278	4.5978
SE($\hat{\beta}_3$)	0.0175	0.0157	0.6423

TABLE 7. Iterative principal component and Stein estimates and associated asymptotic standard errors for lake trout data ($N=32$).

	pc(-1)	pc(-2)	Stein($c=0.77$)
$\hat{\beta}_0$	3.8311	−6.3505	−179.6000
$\hat{\beta}_1$	1.5139	0.2607	24.9259
$\hat{\beta}_2$	−2.9043	0.6635	35.1343
$\hat{\beta}_3$	0.0629	0.0355	−4.8637
SE($\hat{\beta}_0$)	8.2051	3.9684	98.1859
SE($\hat{\beta}_1$)	0.9215	0.1637	12.8122
SE($\hat{\beta}_2$)	2.5853	0.4164	20.0781
SE($\hat{\beta}_3$)	0.0302	0.0223	2.6320

natural units, i.e. Tables 4–5 and Fig. 1–2 provide $\hat{\beta}_R$ and SE($\hat{\beta}_R$).

The results of the iterative principal components analysis and Stein method are given in Tables 6 and 7. Tables 6–8 use the uncentered and unscaled $\hat{\beta}_s^{pc}$ and SE($\hat{\beta}_s^{pc}$). The estimates obtained by deleting one component are not satisfactory in terms of the signs of the coefficients. The second set of estimates, obtained by deleting two components, is more satisfactory. Note that the estimates obtained by Reckhow et al. (1987, their table 4) are quite close to these except for the coefficient for alkalinity. In contrast to Reckhow et al., we did not find close similarity between principal component estimates and the ridge estimates. In particular, the signs of the coefficients were not consistent between the two methods. Further, the ridge standard errors for the estimates still seem inflated. The addition of the interaction term is not supported. Thus this analysis would suggest that the model is not appropriate.

TABLE 8. Estimated rate of a change in probability of occurrence (at $\hat{\pi}_i = .5$) per unit change in explanatory variable ($\delta X_i = 1$) for various estimation techniques using trout data.

Brook trout $\frac{\delta\hat{\pi}_i}{\partial X_i}$	ML	Ridge (0.001)	Ridge (0.002)	pc (−1)	pc (−2)	Stein
pH	2.298	0.883	0.685	0.558	0.073	1.789
ln (Ca)	2.002	−0.270	−0.245	−0.745	0.138	1.559
pH * ln (Ca)	−0.372	−0.073	−0.036	0.013	0.009	−0.289
Lake trout $\frac{\delta\hat{\pi}_i}{\partial X_i}$						
pH	8.096	0.623	0.469	0.378	0.065	6.231
ln (Alkalinity)	11.411	−0.203	−0.389	−0.726	0.166	8.784
pH * ln (Alkalinity)	−1.579	−0.045	−0.016	0.016	0.009	−1.216

The Stein method does not seem to produce good results as the coefficients are too large relative to single variable models. We therefore cannot recommend it as a technique. Table 8 provides estimates of the rate of change in the probability of occurence of trout species for a given unit change in a specific explanatory variable (i.e. if x_i is changed by one unit, what is the resulting change in the probability of occurrence). It is interesting to note that except for maximum likelihood and Stein methods, the estimated changes are similar across species for a given method. However, there are considerable differences between the methods. Although we do not think that any of the methods is appropriate for this data, the method that incorporates all the variables and seems best is the principal components method, deleting two components associated with the smallest eigenvalues.

From a modelling point of view, notice that none of the approaches are totally satisfactory. Most of the coefficients are only marginally significant and some of the coefficients are not significant (at 0.05 significance level). Furthermore for the models given in Table 1, the maximum likelihood estimated standard errors are all deemed infinite (Hauck and Donner 1977) except for both intercepts and the pH coefficient in the brook trout model. Harrell (1986) deems the standard errors infinite, in the SAS software Proc Logist procedure, if the absolute value of a parameter estimate is greater than or equal to five divided by the range of the corresponding explanatory variable, and its standard error is greater than or equal to 15 divided by the range. The trout examples coefficients have such a limited range. In addition, the WVIFs and the condition numbers in Tables 2–3 indicate severe ill-conditioning of the information matrix, and hence an unstable inverse. The approaches given suggest that either variables need to be deleted or the model needs to be changed.

Discussion

The methods described above are useful for diagnosing possible problems in logistic regression. The diagnostics are quite effective at detecting multicollinearity and indicating which variables are involved. The logistic ridge, principal component, and Stein methods provide reasonable alternative parameter estimates to that of maximum likelihood, especially when weighted multicollinearities are detected and explanatory variables are not deleted. Evidence from applications to acid rain and other data sets indicates that the principal components and ridge methods generally give reasonable results. The Stein approach does not shrink the estimates enough and we cannot recommend it as a useful procedure. Choice of which method is better in any application depends on the purpose of the model. Models in which good parameter estimates are required should be assessed differently from models in which good prediction is required. With complex data, one need not expect a single model to be the best for all purposes.

The ridge, principal component, and Stein methods described here for logistic regression can substantially reduce the variance of the estimated coefficients and prediction variance for future observations outside the mainstream of weighted multicollinearity (i.e. future observations having levels of the explanatory variables not among the patterns of data when the columns of $\hat{S}$ are plotted). The principal components method presented here uses an approach for deletion of components based on the magnitude of the eigenvalues and assessment of fit of the reduced model. Other approaches are possible, i.e. prediction. The principal component approach given here differs from that of Reckhow et al. (1987). In their approach, a principal component analysis of $X_p' X_p$ was done, then logistic regression on leading components. This approach, while successful in many cases, ignores the underlying model and may lead to difficulties. In fitting models, assessment and adjustment for multicollinearity is only one aspect of the modelling process. Other concerns, such as influential values (Pregibon 1981) and assessment of prediction are also impotant and need to be addressed. Lastly, the biased estimation techniques described in this paper can be used in the broader framework of the generalized linear model as described in Marx and Smith (1990) and Marx (1988).

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Smolting Status of Downstream Migrating Atlantic Salmon (*Salmo salar*) Parr[1]

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Significant numbers of Atlantic salmon (*Salmo salar*) parr have been observed to accompany seaward migrating smolts in some rivers. Downstream migrating parr in Freshwater River Newfoundland, have lower salinity tolerance and branchial Na^+–K^+ ATPase activity than smolts. However, moisture content in downstream migrating parr was similar to that of smolts; both groups had higher mean moisture content than nonmigratory parr. Mean plasma sodium concentration was significantly higher than in smolts, but somewhat lower than in nonmigratory parr. Elevated moisture levels and slightly depressed plasma sodium concentrations are consistent with the view that seaward movement is associated with osmoregulatory imbalance in both parr and smolts. Brackish water found in estuaries appears important for gradual development of salinity tolerance in these migrants.

Dans certaines rivières de Terre-Neuve, on a observé un nombre important de tacons de saumon de l'Atlantique qui accompagnaient des saumoneaux en migration vers la mer. Les tacons dévalants de la rivière Freshwater ont une plus faible tolérance à la salinité et activité de la Na^+–K^+ ATPase branchiale que les saumoneaux. La teneur en humidité des tacons dévalants était toutefois semblable à celle des saumoneaux; les deux groupes montraient des teneurs moyennes en humidité plus élevées que les tacons non migrateurs. La concentration moyenne de sodium dans le plasma des premiers était nettement plus élevée que chez les saumoneaux mais légèrement inférieure à celle des tacons non migrateurs. Des teneurs élevées en humidité et une concentration légèrement diminuée de sodium dans le plasma s'accordent avec la théorie selon laquelle la dévalaison est associée à un déséquilibre osmorégulatoire chez les tacons et les saumoneaux. Les eaux saumâtres des estuaires semblent importantes au développement graduel de la tolérance à la salinité chez ces migrateurs.

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During the freshwater stages of the life cycle, young Atlantic salmon (*Salmo salar*) usually occupy flowing freshwater and are characterized by behavioral and physiological traits adaptive to such habitat. Juveniles tend to maintain contact with the substrate and resist downstream displacement by adopting appropriate rheotactic behavior. Maintenance of feeding territories, from which conspecifics are aggressively excluded, helps salmon parr to hold station within the flow (Keenleyside 1962; Keenleyside and Yamamoto 1962; Thorpe 1982). Salmon at this stage of development are hyperosmoregulators, i.e. they maintain internal solute concentrations at higher levels than are present in the external medium. Freshwater salmon are therefore faced with the challenge of conserving internal solutes while at the same time eliminating large amounts of body water.

Recent communications report that, in some instances, significant numbers of apparently presmolt salmon accompany smolts in the spring emigration from freshwater (Shears 1985; Chadwick and Léger 1986; Cunjak et al. 1989). In addition, several authors have reported the occurrence, during the summer months, of presmolt Atlantic salmon in estuaries with salinities as high as 30‰ (Huntsman 1945; Saunders 1960; Power and Schooner 1966; Shears 1985; Hutchings 1986; Cunjak et al. 1989). These salmon are smaller than smolts and retain the characteristic appearance of river-dwelling parr. As it is well established that salmon parr have low salinity tolerance relative to smolts (Huntsman and Hoar 1939; Parry 1960), the adaptive value of movement to these areas is unclear. In this report we describe the smolting status of downstream migrating Atlantic salmon parr in a small river in Newfoundland.

Methods

Study Area

Freshwater River is a small third order stream situated in the southeastern Avalon Peninsula, Newfoundland, Canada (Fig. 1). An impassible waterfall at the stream mouth prevents the return of fish that may descend to the sea. Nonanadromous brook charr (*Salvelinus fontinalis*) is the only naturally occurring salmonid present. Since 1985 adult anadromous salmon from nearby Biscay Bay River have been transferred each summer to Freshwater River and prevented from returning to the sea by a fence erected approximately 1 km upstream from the waterfall. These introduced spawners have reproduced successfully and are the founders of the salmon population. A

[1]Contribution No. 81 from the Ocean Sciences Centre.

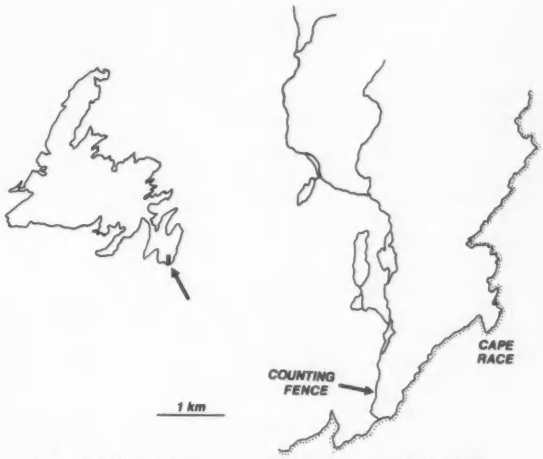

FIG. 1. Freshwater River, Avalon Peninsula, Newfoundland.

counting fence located a short distance upstream from the waterfall has been used to monitor downstream migrating salmon since 1986. Significant numbers of apparently migrant parr move through the fence during the spring at the time of the smolt run (R. J. Gibson, Department of Fisheries and Oceans, Box 5667, St. John's Nfld. A1B 5X1, pers. comm.).

Sampling

Seaward-migrating salmon parr and smolts were sampled at the counting fence on 5 May 1988. Salmon with fork length of 13 cm and below with distinct parr marks were classified as parr while silvered fish greater than 13 cm fork length were classified as smolts. A sample of stream dwelling parr was electrofished several hundred metres upstream of the fence.

Salmon were killed with a blow to the head, weighed, measured, sexed, and blood samples were collected from the severed caudal peduncle. The gill system was removed, washed in ice cold 250 mM sucrose/5 mM EDTA, immersed in fresh sucrose/EDTA and frozen on dry ice. Samples were held at −70°C until further processing (96 h maximum). Gills were thawed, filaments were trimmed from the arches, homogenized, and the activity of Na^+–K^+ ATPase determined according to the method described by Johnston and Saunders (1981). Blood samples were collected in heparinized tubes and centrifuged. Plasma was drawn off and sodium concentration determined using an IL443 flame photometer. Plasma chloride levels were measured using a Corning 920M chloride meter. The salmon carcasses were dried to constant mass at 80°C to determine moisture content.

On 25 May 1989 salinity challenge tests (Clarke and Blackburn 1977) were performed on migrant parr and smolts collected at the counting fence. Salmon were placed directly into aerated seawater (32‰) at the same temperature as the river water (16°C) in a 60–L plastic tank. Although no attempt was made to maintain constant temperature, it did not exceed 16°C. Survivors were removed after 24 h, weighed, measured, sexed, and bled as previously described. Freshwater controls were held in a pen in the stream during this period.

Statistical comparisons of means were made using Student's *t*-test or one-way analysis of variance. Where heterogeneous variances were detected (Bartlett's Test), log transformed data were used in statistical procedures. Statistically significant differences detected by ANOVA were isolated using the Student-Newman-Keuls Test (Sokal and Rohlf 1969).

Results

Mean Na^+–K^+ ATPase activities were similar in parr electrofished from the river and parr captured at the counting fence (Table 1). In both groups mean activity levels were approximately 17 μmoles P_i·mg protein^{-1}·h^{-1}. The mean Na^+–K^+ ATPase activity observed in smolts was more than twice that measured in parr. Differences in activity levels between smolts and both samples of parr were significant ($p<0.01$).

Mean plasma sodium and chloride concentrations are also shown in Table 1. Smolts were found to have significantly lower plasma sodium levels than parr sampled in the river ($p<0.01$) which in turn were not significantly different from parr sampled at the counting fence ($p>0.05$). Similar results were found for mean plasma chloride concentrations ($p<0.05$).

Significant differences were found in mean moisture content of parr electrofished in the river and parr and smolts captured at the counting fence (Table 1). River dwelling parr contained less water than downstream migrating parr ($p<0.05$) and smolts ($p<0.01$).

Results of the salinity challenge experiment are consistent with gill Na^+–K^+ ATPase activities (Table 2). No mortality occurred in either freshwater group. In the seawater treatment, 9 of 12 parr died within 24 h; no mortality occurred among seawater challenged smolts. Two of the three surviving parr were experiencing severe hydromineral imbalance at the end of the exposure period when blood samples were drawn. Mean plasma sodium levels in these three parr was much higher than in the freshwater controls, but Student's t-test did not detect a significant difference in mean values due to the large variance in the former group (however, comparison of these means using the nonparametric Mann-Whitney test indicates a significant difference; $p<0.01$). In contrast to the parr, all smolts exposed to 32‰ salinity survived the 24-h period and appeared healthy and active. Plasma sodium was also elevated in the group exposed to seawater relative to the freshwater smolts ($p<0.05$); however mean values were not as high as those of parr exposed to seawater.

Discussion

These results suggest that Atlantic salmon parr emigrating from Freshwater River at the time of the annual smolt run are not physiologically equipped for rapid acclimation to seawater. Mortality was high among parr challenged in the high salinity condition, and two of the three surviving parr were suffering severe distress after 24-h exposure. Smolts exposed to this treatment showed no outward symptoms of distress and the moderate elevation in mean plasma sodium relative to freshwater controls indicates they were capable of successful acclimation to seawater.

Downstream movement and presence of salmon parr in estuaries have been documented by several workers (Huntsman and Hoar 1939; Huntsman 1945; Saunders 1960; Power and Schooner 1966; Cunjak et al. 1989) although their biological significance and the motivating factors involved are unclear. Based upon enumeration of parr and smolts leaving Western Arm Brook, Newfoundland, over a 13-yr period, Chadwick and Léger (1986) found a significant correlation between the num-

TABLE 1. Mean levels (±2 SE) of gill Na^+–K^+ ATPase activity, mean plasma Na^+ and Cl^- concentrations and moisture content of Atlantic salmon parr and smolts sampled in Freshwater River and at the smolt counting fence. For the Student-Newman-Keuls test, means underscored by the same line are not significantly different.

	(1) Parr (River)	(2) Parr (Fence)	(3) Smolt	Student-Newman-Keuls test
Na^+–K^+ ATPase[a]	17.5±3.3	17.2±3.2	44.5±4.7	1 2 3
Plasma Na^+[b]	149.5±1.7	146.2±3.7	140.7±3.9	1 2 3
Plasma Cl^-[b]	135.2±2.3	138.0±1.8[c]	128.4±5.0	1 2 3
Moisture content[d]	76.2±0.5	77.5±0.6	77.6±0.6	1 2 3
N	10	5	16	

[a]μmoles P_i·mg protein^{-1}·h^{-1}.
[b]Meq·L^{-1}.
[c]$N=4$.
[d]Percent.

TABLE 2. Mortality, mean fork length, and plasma sodium concentration of downstream migrating salmon parr and smolts after 24-h salinity challenge test.

		Mortality (%)	Fork length (cm)	*N*	Plasma sodium (Meq·L^{-1} ± 2 SE)
Seawater[a]	Parr	75	10.5	12	211.0±39.4[b]
	Smolt	0	14.0	10	172.2±8.66
Freshwater	Parr	0	10.7	14	155.7±1.90
	Smolt	0	14.5	11	159.0±2.76

[a]32‰.
[b]Mean of three parr surviving 24-h exposure.

ber of parr migrating and the number of 3-yr-old salmon present in the system prior to smolt migration. In four consecutive years the outmigration of parr preceeded that of smolts. This suggests that crowding results in displacement of parr out of the river. Further support for the role of density-dependent factors comes from behavioral studies. Salmon parr aggressively defend feeding territories against conspecifics, especially under conditions of high current velocity (Wankowski and Thorpe 1979), and it has been observed in several salmonids that subordinate individuals displaced by agonistic interaction with more dominant fish are inclined to move downstream (Chapman 1962; Gibson 1981; McNicol and Noakes 1981). This sort of social interaction, if widespread in the spring prior to smolt migration, i.e. when densities are high, may result in a net downstream movement of parr. This is consistent with the observation that these parr represent relatively slowly growing members of their cohorts (Chadwick and Léger, 1986) and are therefore likely to be unsuccessful in competition for feeding territories with larger, more rapidly growing salmon.

On the other hand it is unlikely that density dependent factors and high river discharge are the only factors important in downstream movement of parr. The intensity of agonistic interaction between salmon parr decreases as food availability increases (Symons 1968; Wankowski and Thorpe 1979). During the spring when downstream movement occurs, food availability within rivers is relatively high, therefore a decrease in aggressive behavior would be expected at this time. Parr would be expected to maintain position in the stream under these conditions although it is clear that many do not.

Downstream movement of parr may also be related to habitat change that young salmon undertake as they develop in the river. As food requirements (and swimming ability) increase with growth they seek deeper water and higher current velocity (Thorpe 1988). Since these habitat characteristics are likely to be found downstream of the shallow areas occupied by very small salmon, movement would naturally tend to be directed downstream (Thorpe 1982). This sort of movement, if concentrated during the spring, could result in some parr leaving the river and entering the estuary.

The parr–smolt transformation has been traditionally regarded as a series of alterations that pre-adapt juvenile salmon to conditions they will encounter in the sea. Implicit in this view is the notion that salmon are primarily freshwater fish and are capable of life in the marine environment by virtue of a series of secondary adaptations which are expressed during the smolting process and remain functional throughout the marine phase of the life cycle. Thorpe (1982) has challenged this view, and argues that salmonids are primarily marine fishes that have secondarily occupied freshwater. Accordingly the parr–smolt transformation is regarded not as pre-adaptation to the marine environment, but rather as the gradual breakdown of adaptations for life in the dilute freshwater medium and a reassertion of the ancestral physiological state. This breakdown is manifested by reduced plasma electrolytes and elevated body water content and results in reduced swimming activity and therefore downstream displacement (Thorpe 1988). Viewed this way, seaward movement of smolts does not constitute directed migration but rather is an involuntary effect of deteriorating adaptation. Our results tend to support the view that downstream movement is associated with, and may result from, hydromineral imbalance. Plasma sodium and chloride, gill Na^+–K^+ ATPase activity and body water content were significantly different in Freshwater River smolts and apparently nonmigrant parr. It is also of interest that parr sampled in the river had significantly lower body water content than parr caught at the counting fence. Mean plasma sodium concentration was also lower in the downstream moving parr relative to parr sampled in the river, although the difference was not statistically significant. Thus there is some suggestion that downstream movement is associated with hydromineral imbalance in both parr and smolts although the imbalance is more severe in smolts.

Exposure to intermediate salinities encountered in estuaries is probably important for the gradual development of hypoosmoregulatory ability in parr. Whether any of these salmon later move out to sea is not known. Shears (1985) and Cunjak et al. (1989) have observed that at least some of these estuarine parr return to the river after the smolt emigration, however the proportion which do so is not known. Similarly, Hutchings (1986) reported that some male Atlantic salmon parr which left Wings

Brook and entered the estuary in the spring returned the following autumn as sexually mature parr. However, attempts by Power and Schooner (1966) and Shears (1985) to recapture tagged estuarine parr during the summer months were largely unsuccessful, suggesting that either mortality is high or that they leave the estuaries. While it is clear that many do not immediately die, osmoregulatory performance is probably a limiting factor especially in estuaries which do not provide parr with sufficient low salinity refuge areas. It is relevant to note that rivers in which downstream migration of parr has been observed are associated with reasonably large estuaries wherein parr could find salinities considerably lower than that of seawater. Parr whose hypoosmoregulatory capacity is not fully developed could find temporary relief from high salinity in these estuaries, and gradually develop greater salinity tolerance. Biscay Bay River, the river from which the Freshwater River salmon are derived, also drains into a large estuary. It may be that salmon stocks in rivers with large estuaries are more predisposed to downstream movement at the parr stage than stocks present in rivers that do not drain into sizeable estuaries. Further research is needed to determine the degree to which these estuarine parr contribute to the overall salmon production of their natal rivers.

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Denitrification in Sediments from the Hyporheic Zone Adjacent to a Small Forested Stream

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Denitrification was assayed by the acetylene blockage technique in hyporheic sediments. Samples were obtained along transects perpendicular to the stream at two sites: (1) the base of a slope dominated by old-growth redwood and (2) the base of a slope dominated by alder regenerating from a clearcut in 1965. Denitrification was evident at in situ nitrate concentrations at all locations tested. Activity was stimulated by nitrate but nitrate plus glucose had no additional effect. Denitrifying potentials increased with increasing distance from the stream channel. Dissolved oxygen was 100% of the concentration expected in equilibrium with the atmosphere in water obtained from monitoring wells immediately adjacent to the stream but was as low as 7% of the expected value in water 11.4 m inland. Both nitrate and dissolved organic carbon decreased over summer in wells at the base of the alder-forested slope. A 48-h injection of nitrate-amended stream water into hyporheic water 8.4 m inland stimulated nitrous oxide production in the presence of acetylene. Nitrous oxide was generated as nitrate and acetylene were co-transported to a well 13 m down-gradient. The acetylene-block experiments coupled with the chemistry data suggest that denitrification can modify the chemistry of water during passage through the hyporheic zone.

Des essais de dénitrification ont été effectués par la technique de blocage à l'acétylène dans des sédiments hyporhéiques. Des échantillons ont été prélevés à deux endroits dans des transects perpendiculaires au cours d'eau : (1) à la base d'une pente dominée par des séquoias mûrs et (2) à la base d'une pente dominée par de jeunes aulnes repeuplant un parterre de coupe de 1965. La dénitrification était évidente pour des concentrations de nitrate du milieu naturel à tous les endroits analysés. L'activité était stimulée par le nitrate, mais l'addition de glucose au nitrate ne provoquait aucun autre effet. Les potentiels de dénitrification augmentaient avec l'éloignement du cours d'eau. L'oxygène dissous en équilibre avec l'atmosphère correspondait à 100% de la concentration dans l'eau prévue et mesurée dans des puits immédiatement adjacents au cours d'eau, mais atteignant à peine 7% de la valeur escomptée dans des eaux situées à 11,4 m à l'intérieur des terres. Le nitrate et le carbone organique dissous diminuaient pendant l'été dans les puits situés à la base de la pente boisée d'aulnes. Une injection d'eau additionnée de nitrate dans des eaux hyporhéiques, situées à 8,4 m dans les terres, pendant une période de 48 heures, a stimulé la production d'oxyde nitreux en présence d'acétylène. L'oxyde nitreux était produit sous forme de nitrate et l'acétylène était transporté conjointement à un puits situé à 13 m en bas du gradient. D'après les expèriences de blocage à l'acétylène combinées aux données chimiques, la dénitrification peut modifier les propriétés chimiques de l'eau lorsqu'elle circule dans la zone hyporhéique.

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In upland watersheds, a variable fraction of the total stream discharge is exchanged between open-channel stream water and interstitial flows beneath and parallel to the stream channel (Bencala 1984; Kennedy et al. 1984; White et al. 1987; Triska et al. 1989). These subsurface zones, characterized by the direct and frequent contact of both stream and groundwater, comprise a dynamic and viable stream habitat — the hyporheic zone (Hynes 1983). At the base of the hyporheic zone, an irregular boundary is formed at the groundwater–stream water interface that constitutes the boundary of the stream ecosystem (Triska et al. 1989). Within the stream ecosystem, bidirectional exchange of nutrients can occur between stream water and the hyporheic zone.

The supply of dissolved nutrients to stream biota can be affected by the exchange between stream water and groundwater. For example, well-oxygenated hyporheic sediments foster nitrifying bacteria that can generate nitrate in excess of the biotic demand in the channel (Triska et al. 1990). Oxygen dissolved in stream water may be gradually depleted by physical, chemical, or biological processes as stream water is transported through the hyporheic zone. Exchange of oxygen between stream and subsurface habitats is important because oxygen availability commonly controls the oxidation and reduction of nitrogen compounds by bacteria (Knowles 1982). In areas of the hyporheic zone where dissolved oxygen is low, zones of denitrifying activity may be established.

In this study, we directly examine sediments from the hyporheic zone adjacent to a small forested stream for denitrification. We hypothesize that these sediments are potential sites of microbial denitrification and may act as a nitrate sink prior to reentry of subsurface water into the channel.

Materials and Methods

Study Site

Little Lost Man Creek is a pristine drainage near the town of Orick in northwestern California (Fig. 1). The stream flows

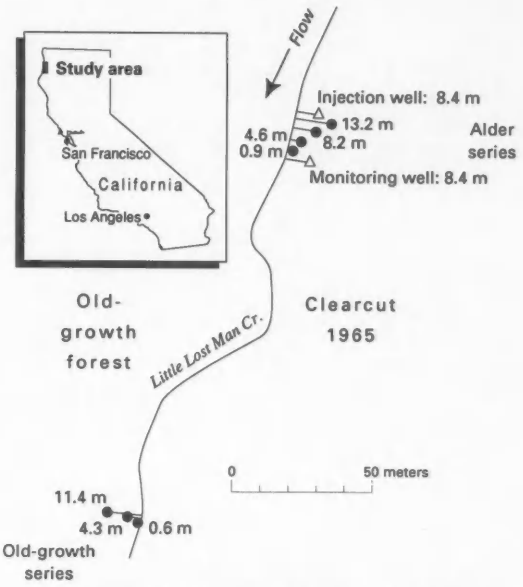

FIG. 1. The study site at Little Lost Man Creek, California.

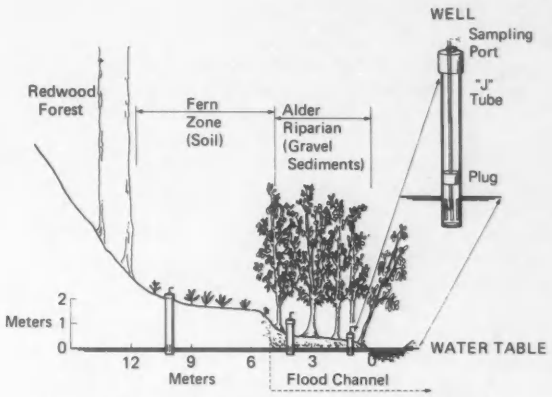

FIG. 2. A schematic cross section of the old-growth wells.

through Redwood National Park and is a third-order pool-and-riffle channel draining a long narrow coastal valley with steep slopes. The watershed area is 9.4 km^2 and ranges in altitude from 24 to 695 m. The overall channel gradient is 0.066%. The high permeability of the stream bed results in significant interflow during transport. Discharge ranges between 0.01 and 0.04 $m^3 \cdot s^{-1}$ during base flow. Approximately 90% of the vegetation is old-growth coastal redwood forest (*Sequoia sempervirens*) that includes associated Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*). Dominant riparian vegetation is red alder (*Alnus rubra*). About 10% of the watershed was clearcut in 1965. This study was done along a 220-m reach of Little Lost Man Creek that spans the clearcut and old-growth forest.

Sampling

In 1983, seven monitoring wells were placed in two distinct stream-side habitats across the riparian zone. One well series was placed at the base of a forested slope clearcut in 1965. Red alder has invaded the clearcut (Fig. 1). A second well series was located at the base of an old-growth coastal redwood forest typical of the watershed. The wells were located along transects perpendicular to the stream channel as far as 13.2 m inland.

Water samples were obtained from monitoring wells that were cased with 15-cm diameter polyvinyl chloride (PVC) pipe screened for a distance of approximately 20 cm below the water table at low flow (Fig. 2). The PVC casing was open at the base and capped above the ground. An aluminum plug (14 cm diameter) was positioned immediately above the water table to reduce gas exchange with the atmosphere. Water was sampled with peristaltic pumps through 0.64-cm diameter stainless steel tubing positioned in the center of the casing. For inorganic nutrients, water was pumped through a 142-mm diameter in-line filter (0.45-μm pore size Metricel filters, Gelman Sciences, Inc., Ann Arbor, MI[1]) into polyethylene bottles rinsed with sample three times. For dissolved organic carbon (DOC), water was pumped through a 142-mm diameter in-line glass fiber filter (Gelman A/E) housed in a stainless steel holder into glass bottles. Samples for DOC were subsequently filtered through 47-mm diameter silver filters into glass ampules. The glassware and glass fiber filters were combusted for 10 h at 450°C prior to sampling. Dissolved oxygen was measured with an oxygen probe in a polycarbonate cylinder. Well water was delivered continuously to the bottom of the cylinder until three to five times the capacity of the cylinder had overflowed and the oxygen reading had stabilized. Dissolved gases (acetylene and nitrous oxide) were extracted from water using the syringe shaking method of Rudd et al. (1974). Gas and nutrient samples were collected in triplicate.

Subsurface riparian sediment was collected from the cased wells by using a small plastic scoop at the end of a 1.8-m pipe. The cap, stainless steel tubing, and aluminum plug were removed to obtain a sample. Fresh sediment material was transferred to a nearby laboratory in Zip-lock bags. The upper 6 to 10 cm of stream sediment was collected in the same fashion after larger stones were removed from the surface. Fresh sediments were collected for each incubation.

Incubations

Denitrification was assayed by the acetylene blockage technique (Balderston et al. 1976; Yoshinari et al. 1976). Approximately 5 mL of sediment (9.6 g wet weight) was added to 20-mL glass bottles by using a wide-bore (1 cm) plastic syringe that had the end cut off. The sediment was slurried with 5 mL of water obtained from the same well. The bottles were flushed for 15 min with oxygen-free nitrogen and then sealed with recessed butyl rubber stoppers. Acetylene was generated from calcium carbide-saturated water and was added (2 mL) by syringe to the headspace of each bottle. The bottles were incubated on a shaker table at 18°C for 15 min after which 100-μL subsamples of the headspace were removed for nitrous oxide analysis. Headspace concentrations were corrected for nitrous oxide dissolved in the aqueous phase (Flett et al. 1976) using a Bunsen coefficient of 0.69.

[1]Use of brand names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

The effects of various amendments on denitrification also were analyzed. To differentiate carbon limitation from nitrate limitation, additional bottles were set up with acetate, glucose, nitrate, and nitrate plus acetate or nitrate plus glucose. For each substrate, 100 μL of 60-mM stock solution was added to each bottle. Stock solutions were flushed with oxygen-free nitrogen prior to amendment. Some sediment slurries were incubated with air in place of oxygen-free nitrogen and helium in place of acetylene. Denitrification also was tested in unfiltered water samples enriched with nitrate and glucose (1 mM final concentration each). Fifteen millilitres of water were added to 20-mL bottles which were incubated anaerobically as described above.

Nitrate Injection Experiment

Stream water amended with nitrate was injected directly into subsurface water 8.4 m inland from the stream channel to assess the potential for denitrification by the indigenous bacterial population. A field modification of the acetylene blockage technique was used to inhibit bacterial reduction of nitrous oxide co-transported with acetylene. The injectate was prepared in a polyethylene carboy at the study site by adding 150 g of $NaNO_3$ to 24 L of stream water. The solution was injected through a metering pump (Fluid Metering Inc., Oyster Bay, NY) directly into a cased injection well (Fig. 1) at a rate of 8 $mL \cdot min^{-1}$. Acetylene (99.6 minimum purity, Matheson Gas Products, Newark, CA) was co-injected directly into the water in the cased injection well at a rate of approximately 10 $mL \cdot min^{-1}$. The volume of water in the injection well was approximately 3 L and the subsurface velocity was 1–2 $m \cdot h^{-1}$. The subsurface velocity and flow path were determined in a 1984 injection experiment with sodium bromide at the alder-forested site (G. W. Zellweger, U.S. Geological Survey, 345 Middlefield Rd., MS 496, Menlo Park, CA 94025, unpubl. data). Water samples for nitrate, dissolved nitrous oxide, and dissolved acetylene were collected every 4 to 6 h in the monitoring well approximately 13 m down-gradient. The injection ran for 48 h.

Analytical Procedures

Oxygen concentration and temperature were measured directly in water samples collected in the field using an oxygen meter (Yellow Springs Model 57, Yellow Springs Instrument Company, Yellow Springs, OH) with a probe mounted on a stirring platform. Nitrate and ammonium were measured on an Autoanalyzer II (Technicon Corp. Inc., Tarrytown, NY) using a cadmium-reduction method and a phenol-hypochlorite method, respectively. Nitrite was subtracted from nitrate + nitrite. DOC (as CO_2) was analyzed on a Beckman model 864 infrared analyzer (Beckman Instruments, Inc., Fullerton, CA) following sample digestion similar to the method of Menzel and Vaccaro (1964). Nitrous oxide and acetylene were determined on a gas chromatograph (Perkin-Elmer Sigma 2000, Perkin-Elmer, Norwalk, CT) equipped with a flame-ionization detector and a ^{63}nickel electron capture detector. The electron capture detector was equipped with two Poropak® R stainless steel columns (244 cm long, 0.22 cm inside diameter) split by an eight-port backflush valve to vent the acetylene gas. The carrier was ultra high purity (UHP) oxygen-free nitrogen at a flow rate of 30 $mL \cdot min^{-1}$. The flame ionization detector was equipped with one Poropak® column (20% Q, 80% N; 305 cm long, 0.22 cm inside diameter) with UHP helium as the carrier gas (30 $mL \cdot min^{-1}$).

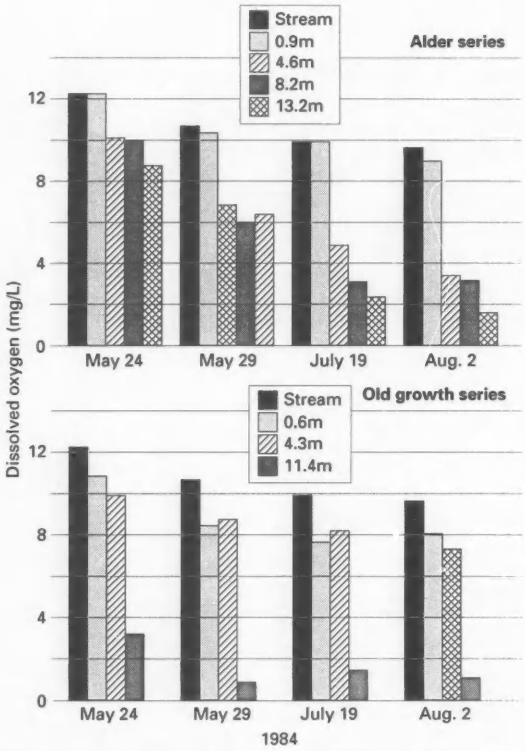

FIG. 3. Dissolved oxygen obtained from the alder and old-growth wells during base flow.

TABLE 1. Temperature (°C) and percentage saturation (%) of dissolved oxygen.

Date	5/24/84		5/29/84		7/19/84		8/14/84	
Distance (m)	(°C)	(%)	(°C)	(%)	(°C)	(%)	(°C)	(%)
				Alder well series				
Stream	9.5	107	10.0	94	12.0	91	13.3	91
0.9	9.2	106	10.0	91	12.0	91	14.8	88
4.6	9.8	88	11.2	62	12.8	45	13.8	32
8.2	10.0	87	11.5	54	11.5	28	12.0	28
13.2	10.0	78	10.9	57	11.0	21	11.5	14
				Old-growth well series				
Stream	10.3	107	12.2	94	15.5	91	15.0	91
0.6	9.4	94	10.0	74	14.2	75	13.8	78
4.3	9.4	85	10.1	77	13.0	77	13.4	69
11.4	10.0	28	10.1	7	11.2	13	12.0	9

Results

Chemical Gradients

Dissolved oxygen decreased laterally from the stream channel and temporally throughout summer (Fig. 3). Dissolved oxygen in the alder wells ranged from 12.2 $mg \cdot L^{-1}$ in stream and bankside water (0.9 m) in May to less than 2.0 $mg \cdot L^{-1}$ in the farthest well inland (13.2 m) in August. Dissolved oxygen in the stream and most bankside wells was 88 to 107% of the concentration expected in equilibrium with the atmosphere on

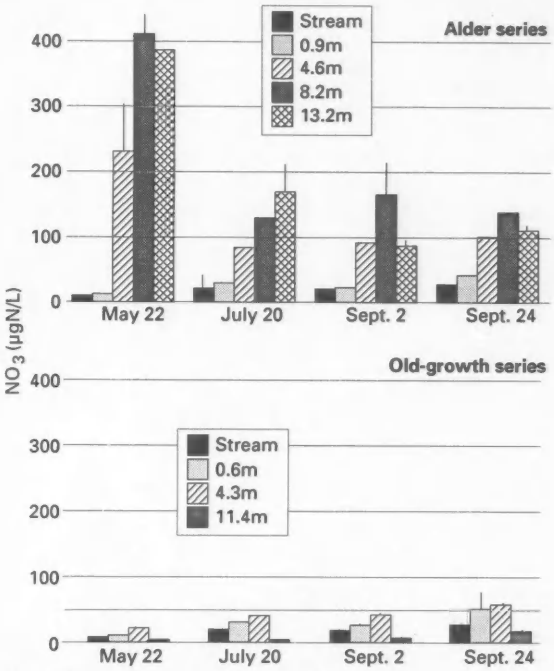

FIG. 4. Nitrate concentration obtained from the alder and old-growth wells during base flow. Error bars are ± 1 SD.

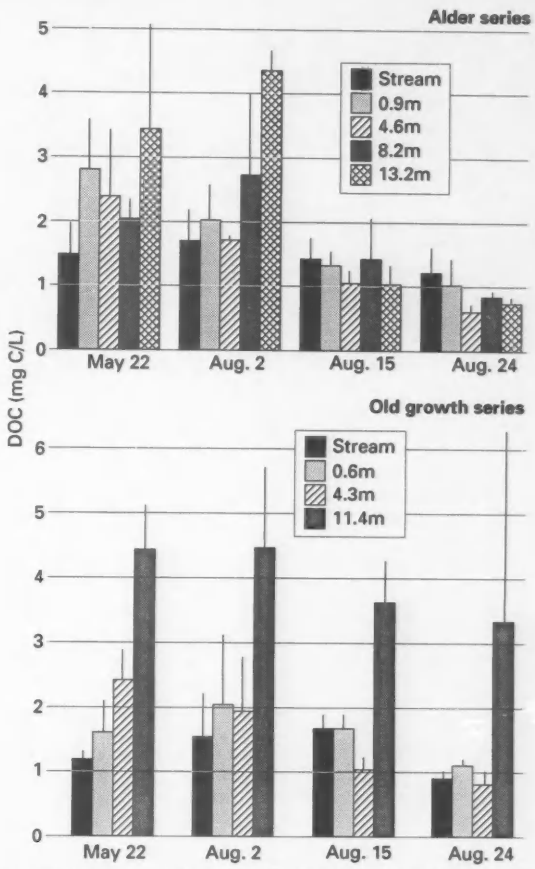

FIG. 5. Dissolved organic carbon obtained from the alder and old-growth wells during base flow. Error bars are ± 1 SD.

all dates (Table 1). The expected concentration is based on the measured temperature of the water. Dissolved oxygen in water 4.6 to 13.2 m inland was up to 88% of the concentration expected in equilibrium with the atmosphere in May, but was reduced to as low as 14% of the expected concentration by August. Similar results were obtained in the old-growth wells; one exception was in water 11.4 m inland, where dissolved oxygen was significantly below the expected concentration on all dates.

Nitrate concentration in the alder wells ranged from 8 µg $N \cdot L^{-1}$ in the stream to 411 µg $N \cdot L^{-1}$ in water 8.2 m inland in May (Fig. 4). The concentration of nitrate in wells 4.6 to 13.2 m inland was always greater than in the stream or bankside well (0.9 m). While the nitrate concentration of stream water and bankside water roughly tripled by August (11 to 41 µg $N \cdot L^{-1}$), there was a dramatic decrease in the nitrate concentration of water from wells 4.6 to 13.2 m inland over the same time. Both the alder and old-growth series had similar nitrate concentration patterns in water up to 4.6 m from the stream. However, beyond 4.6 m, the old-growth series differed from the alder series in three ways: (1) the range of nitrate concentrations from the stream to the farthest well inland was relatively small compared with the alder transect, (2) nitrate tended to increase rather than decrease throughout the summer as far inland as 11.4 m and (3) the concentration of nitrate in the farthest well inland was always significantly lower than that of stream water.

Dissolved organic carbon generally decreased during summer in the stream and in wells at both transects (Fig. 5). DOC ranged between 0.5 and 2.5 mg $C \cdot L^{-1}$ except in the farthest wells inland at each location (13.2 and 11.4 m), where DOC was approximately 4.5 mg $C \cdot L^{-1}$. In May and early August, DOC was higher in subsurface water compared with the stream. By late August, DOC in well water was higher than that in the stream at only one location, 11.4 m inland at the old-growth series.

Denitrification Assays

Slurries of subsurface sediment and well water obtained 8.2 m inland at the alder well series produced nitrous oxide when incubated in the presence of acetylene at in situ nitrate concentrations (Fig. 6a). Nitrous oxide production was linear up to 3.3 h, in most cases without a time lag. Production of nitrous oxide was stimulated by nitrate addition (1 mM final concentration). Further, addition of a readily utilizable carbon substrate to the incubation flasks (1 mM glucose final concentration) had negligible effect over nitrate alone. Nitrous oxide production by sediment slurries was inhibited by the presence of air in the headspace of the flasks (Fig. 6b); when the headspace was oxygen-free nitrogen, significant nitrous oxide accumulated in the absence of acetylene. Well water incubated without sediment did not produce detectable nitrous oxide at any site (data not shown).

The addition of 1 mM nitrate also stimulated denitrification in subsurface sediment obtained 11.4 m inland at the old-growth

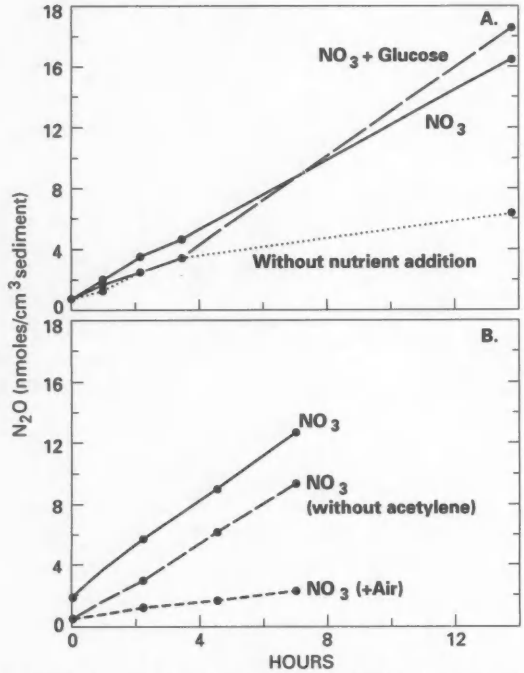

FIG. 6. Nitrous oxide formation in sediment slurries. Sediment was obtained from a cased well 8.2 m inland at the alder well series on two dates in August, 1984. Data points represent individual flasks. Flasks were incubated anaerobically (18°C) with acetylene except where noted. Final concentration of nitrate and glucose 1 mM each.

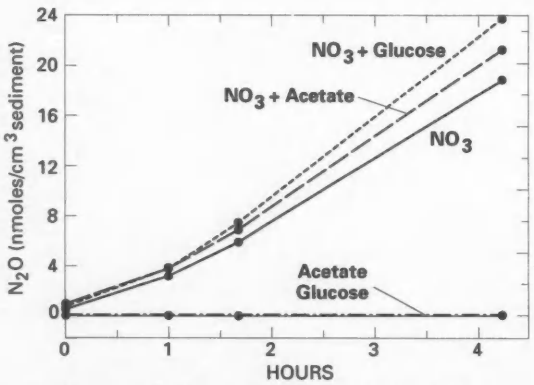

FIG. 7. Nitrous oxide formation in sediment slurries. Sediment was obtained from a cased well 11.4 m inland at the old-growth well series in August, 1984. Flasks were incubated anaerobically (18°C) in the presence of acetylene. Final concentration of nitrate, glucose and acetate 1 mM each.

series (Fig. 7). Neither nitrate plus glucose nor nitrate plus acetate had an effect on nitrous oxide formation over nitrate alone. Glucose- or acetate-amended slurries alone had no effect on denitrification rates.

Denitrifying activity under in situ nitrate and nitrate-amended conditions increased with distance from the stream channel at the alder well series (Fig. 8). Denitrifying activity was about

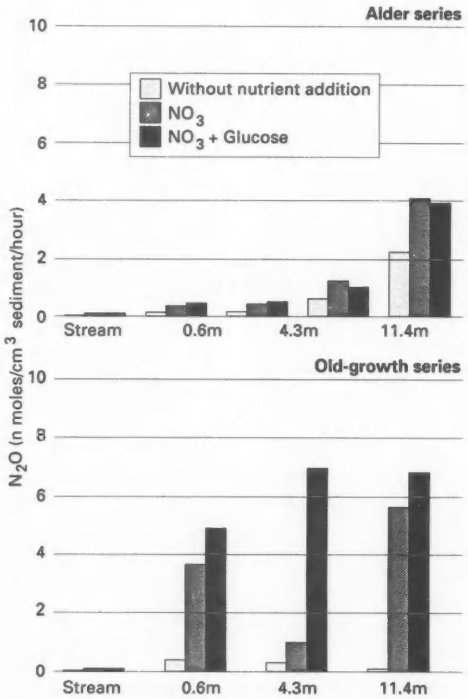

FIG. 8. Spatial gradients of nitrous oxide formation rates under various nutrient conditions in sediments slurries from the alder and old-growth wells. Rates were extrapolated from best fit linear regression of three to four time points from individual flasks.

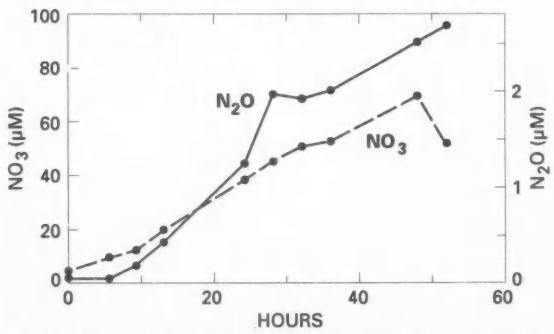

FIG. 9. Nitrate and nitrous oxide concentration 13 meters downgradient of a 48-h co-nitrate plus acetylene injection into a well located 8.4 m inland of the stream. The field experiment was performed in August, 1985.

three-fold higher 13.2 m inland than at the next closest site (8.2 m). Denitrifying activity at in situ nitrate concentrations was low in subsurface sediment from the old-growth series but was enhanced by nitrate addition. At one well, 4.3 m inland, glucose plus nitrate enhanced nitrous oxide formation over nitrate alone.

Field Injection

Data collected during the nitrate injection experiment indicated a close coupling between nitrate arrival and nitrous oxide

formation in the subsurface water (Fig. 9). The leading edge of the injected nitrate travelled approximately 13 m through the subsurface riparian zone in less than 5.5 h. Within 9 h, nitrous oxide was observed. Nitrous oxide accumulated in the presence of acetylene at an increasingly faster rate as nitrate increased from 10 to 40 μM over 24 h (Fig. 9). Acetylene increased from 3.3 to 4.7 μM during the same time (data not shown). Nitrate was approximately 8 times background after 24 h. Nitrous oxide continued to accumulate as nitrate increased from 40 to 70 μM, but at a slower rate. Nitrous oxide dissolved in subsurface water was 55 times higher than background 48 h after its arrival and was rising when sampling was stopped. Both nitrate and acetylene concentrations declined after cutoff.

Discussion

Of all the dissolved inorganic nitrogen species found in headwater streams, nitrate is the most mobile. Its production begins with the decomposition of organic matter, which results in formation of ammonium (Blackburn 1983). In anoxic stream environments, the ammonium can be reassimilated by bacteria. In environments where oxygen is present, the ammonium is oxidized by nitrifying bacteria to nitrate (Schmidt 1982; Blackburn 1983). Because ammonium is readily sorbed onto sediments, the conversion to nitrate provides an easily transported source of nitrogen to stream biota. Plants, algae, and aerobic bacteria can assimilatively reduce nitrate for incorporation into cells (Payne 1973), a process that temporarily retards nitrate transport downstream. Nitrate can also diffuse into sediments. Here, if it encounters heterotrophic microbial activity at the appropriate redox potential, the nitrate is denitrified to dinitrogen by bacteria that use it as an electron acceptor (Firestone 1982). Because dinitrogen can be fixed only by certain bacteria, denitrification is a permanent sink for nitrate transported in streams.

The capacity for soils to denitrify is dependent on the in situ denitrifying enzyme content and the derepression of denitrifying enzyme synthesis (Smith and Tiedje 1979; Tiedje et al. 1982). Tiedje et al. (1982) suggest that carbon is more important than oxygen in determining the denitrifying enzyme content of habitats although oxygen inhibits enzyme activity and represses synthesis of new denitrifying enzymes (Payne 1973). The switch from aerobic to anaerobic or partially anaerobic conditions first eliminates oxygen inhibition then releases the synthesis of denitrifying enzymes (Smith and Tiedje 1979).

In sediments from the stream and hyporheic zone of Little Lost Man Creek, the content of DOC is apparently controlled by the influx of groundwater. Because of the long contact time with watershed soils, groundwater is high in reduced compounds such as DOC, DON, ammonium, and methane, as well as other major ions (Triska et al. 1990). An old-growth well, 11.4 m inland, was probably the only true groundwater well in either transect. Levels of DOC in groundwater are gradually depleted as groundwater seeps into the hyporheic zone. Decreases in concentration result from physical dilution as well as chemical and biological oxidations. The concentration gradients we commonly observe are consistent with reports by Wallis et al. (1981) and Dahm et al. (1987). Wallis et al. (1981) measured higher dissolved organic matter (DOM) concentrations in the saturated groundwater zone than in the surface waters of the Marmot Basin in Canada. Dahm et al. (1987) measured higher DOC concentrations in anaerobic and aerobic zones beneath the channel than in stream water at Sulfur Creek, Oregon. Our observations contrast with Meyer and Tate (1983), who found lower DOC levels in groundwater than in surface water in a second-order stream in North Carolina.

Lateral advection of stream water is probably the main source of oxygen to the hyporheic zone. The combination of ammonium supplied in groundwater and oxygen supplied in stream water produces an environment conducive to the biological oxidation of ammonium to nitrate (Triska et al. 1990). The generation of nitrate is important to biotic communities because nitrate is the primary nitrogen source to periphyton in the channel and an electron acceptor during DOC mineralization in anoxic microsites of the hyporheic zone. The net effect of these metabolic oxidation and reduction reactions is the partial control of nitrate availability downstream.

The content of dissolved oxygen in the hyporheic zone is controlled by a variety of physical, biological, and chemical factors. Physically, the content of dissolved oxygen in water is controlled by temperature. The solubility of dissolved oxygen in water decreases as temperatures rise. This physical control is evident in stream water where the oxygen concentration of the water is close to the value predicted on the basis of temperature alone, falling as temperatures rise over the summer. In contrast, oxygen concentrations of subsurface water up to 13.2 m inland fall well under the concentration expected from water temperature alone, indicating a strong demand for oxygen. One biological sink is microbial mineralization of DOC (including methane), whose concentration decreases as temperatures increase during summer. Secondarily, elevated levels of nitrate in the hyporheic zone compared with the stream indicate that nitrification can also constitute an oxygen sink. Chemically, iron may undergo oxidation in the hyporheic zone as groundwater mixes with stream water (C. N. Dahm, University of New Mexico, Albuquerque, NM 87131; A. S. Maest, U.S. Geological Survey, 345 Middlefield Rd., Menlo Park, MS 496, CA 94025, USA, pers. comm.). The impact of this reaction on oxygen concentration in the hyporheic zone is unknown.

In the absence of oxygen, mineralization proceeds through the reduction of nitrate (Knowles 1982). In order that bacteria use nitrate as a terminal electron acceptor, it is necessary that oxygen be near zero. Although oxygen levels below 1 $mg \cdot L^{-1}$ were detected in the hyporheic zone (7% of the concentration expected in equilibrium with the atmosphere), water was not without oxygen. Measurement of the oxygen status of water provides little direct information as to the oxygen status at microsites. Denitrification may still occur if the supply of oxygen to metabolically-active microsites is reduced both by the amount of water through which oxygen must diffuse (Firestone 1982) and by the intense respiratory activity at metabolically-active microsites (Greenwood 1961; Craswell and Martin 1974). In Little Lost Man Creek, the high oxygen demand as well as the demand for nitrate and DOC over time suggest that a nitrate reducing zone could exist within the hyporheic zone.

When assayed by the acetylene blockage technique, stream and subsurface sediments from the alder riparian series displayed a significant potential for denitrification. The activity was linear for 3 to 4 h, did not display an initial time lag, and was inhibited by the presence of air in the incubation flasks. Smith and Tiedje (1979) attributed the initial constant rate (1–3 h) for denitrification in soils to the activity of pre-existing denitrifying enzymes in the soil microflora. Denitrification potentials in unamended incubation flasks increased significantly with increasing distance from the stream channel, confirming the presence of nitrate-reducing activity in zones of low oxygen tension as far away as 13.2 m from the stream channel.

Denitrifying activity was highest at the most inland site in unamended incubation flasks in spite of sufficient carbon to reduce available nitrate at sites closer to the stream. This pattern was evident even in sediment slurries to which nitrate and glucose were added. The latter conditions have been used to assay denitrification capacity or denitrifying enzyme quantity (Tiedje et al. 1982). Smith and Tiedje (1979) found that the full suite of denitrifying enzymes could be synthesized in aerobic soils under conditions in which they were not fully active. When oxygen was removed from aerobic soils, active denitrification commenced almost immediately. These results suggest that combined hydrodynamic and biotic processes occurring along inland flow paths result in the production and maintenance of high levels of denitrifying enzymes in the bacterial population which are available when the conditions become favorable for denitrification.

In the alder riparian series, denitrification potentials were nitrate-limited but not carbon-limited. Sufficient nitrate was available to support denitrification without the addition of nitrate to incubation flasks. However, enough oxidizable-carbon was present that a further stimulation in denitrification occurred when additional nitrate was added to these flasks. Potentially, the availability of nitrate could control mineralization of DOC during summer base flow should oxygen become depleted.

Denitrifying activity was associated with stream and subsurface solids. The population of denitrifying organisms are, therefore, attached to sediments rather than existing as free-living organisms in the water. An analogous situation was observed in a predominant nitrate-reducing zone of a sand and gravel aquifer receiving treated sewage (Smith and Duff 1988) and this situation is probably not different from other subsurface and surface water systems. Denitrification in the hyporheic zone at Little Lost Man Creek is controlled by water flux from the groundwater table and lateral advection of stream water, whereas in estuarine and coastal sediments it is largely limited by the downward diffusion of nitrate into surface sediments (Oremland et al. 1984; Anderson et al. 1984) or by the rate of water movement past the particulate surfaces of an aquifer (Trudell et al. 1986; Smith and Duff 1988).

There is little direct evidence for denitrification in subsurface water systems. Slater and Capone (1987) measured denitrification in aquifer soil and nearshore marine sediments influenced by groundwater, Smith and Duff (1988) measured denitrification in slurried core material from a fresh water sand and gravel aquifer, and Ward (1985) reported denitrification in nitrate-amended core samples obtained adjacent to a septic tile field. In streams, there is direct evidence for denitrification in surface sediments (Van Kessel 1977; Chatarpaul and Robinson 1979; Swank and Caskey 1982; Cooper and Cooke 1984; Christensen and Sorensen 1988). This study is believed to be the first direct evidence reported for denitrification in the hyporheic zone of a small headwater stream. Prior to this, the only direct measurements of deep sediment metabolism were reported by Grimm and Fisher (1984) for the hyporheic zone below the stream channel. We are currently assessing the denitrifying capacity of stream, hyporheic, and groundwater sediments with regard to seasonal and spatial trends of nutrient chemistry and therefore have not emphasized actual rate measurements.

In this study, the level of oxygen demand, the presence of reduced compounds, and the availability of degradable organic substrates indicated that denitrifying conditions could be established as close as 4.6 m to the stream. Denitrifying potential in these zones was confirmed by incubating subsurface sediment slurries in the presence of nitrate and glucose. Manipulation of dissolved oxygen in slurry experiments can lead to uncertainties as to whether the activity exhibited in flasks is indicative of in situ conditions under ambient nitrate and carbon levels. Some potential limitations were overcome by directly assaying denitrifying activity within the subsurface habitat. Field enrichment with nitrate provided evidence of in situ denitrifying activity as nitrate concentrations were elevated from 5 μM (background) to 70 μM, a level that we have observed in subsurface water subsequent to this experiment. Six hours after nitrate arrived, dissolved nitrous oxide in subsurface water increased 210-fold over the concentration expected in equilibrium with the atmosphere. This was surprising because of the high bed permeability and rapid flow of water (>1 m·h^{-1}) during base flow. Because nitrous oxide dissolved in soil water rapidly degases from solution (Bowden and Bormann 1986), the levels we measured represent only nitrous oxide transported and thus underestimate total in situ production. Nitrate concentration and temperature clearly affect the rate of nitrous oxide formation in denitrification assays (Knowles 1982). While temperature was not measured continuously during this experiment, hyporheic water exhibits minimal temperature flux within short periods. In the four weeks preceeding and following this experiment, hyporheic water ranged from 11.5 to 12°C at this site (11.4 m inland). While preliminary, the data suggest that denitrifying bacteria respond rapidly to short-term nitrate pulses and that hyporheic microbial processes can modify the nitrate concentration of subsurface water.

In summary, denitrification occurred in shallow riparian sediments connected to the stream by the hyporheic zone. Increases of denitrifying potentials with increasing distance from the stream channel also correlated with decreasing oxygen concentrations and increasing concentrations of reduced compounds. Denitrifying activity was associated with stream-channel solids. Therefore, maintenance of a denitrifying capacity is dependent on the flux between groundwater and stream water which, in turn, controls the availability of DOC and generation of nitrate. Geochemical environments favorable to denitrification appear to be established through a combination of hydrodynamic, chemical, and biotic processes. Microbial processes in the lateral hyporheic zone may, thereby, modify the chemistry of water before reemergence from the subsurface.

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Use of a General Dose–Response Model for Rockfish Fecundity–Length Relationships

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Richards, L. J., and J. T. Schnute. 1990. Use of a general dose–response model for rockfish fecundity–length relationships. Can. J. Fish. Aquat. Sci. 47: 1148–1156.

In this paper we describe a general method for determining the relationship between fecundity and another fish attribute, such as size or age. Our methods include linear and logarithmic regression models as special cases and are applicable to a wide range of situations. The model we propose is based on the univariate form of the Schnute–Jensen dose–response model. However, we extend the Schnute–Jensen analysis by describing exact inference regions obtained from likelihood contours, to which we assign nominal probability levels. We also provide a method for obtaining an inference band for the predicted curve. We examine the issue of model adequacy as it relates to fecundity–length data from two rockfish (*Sebastes*) species. We show that the extra complexity of our model is justified, as none of the traditional regression models are appropriate for all three of our data sets. Further, we use inference bands to distinguish fecundity–length relationships for quillback rockfish (*S. maliger*) from two areas, but we are unable to distinguish one of these relationships from a similar relationship for copper rockfish (*S. caurinus*).

Le présent article décrit une méthode générale pour déterminer la relation entre la fécondité et un autre caractère du poisson, comme sa taille ou son âge. Les méthodes incluent des modèles de régression linéaire et logarithmique comme cas particuliers et sont applicables à une vaste gamme de situations. Le modèle proposé repose sur la forme à une variable du modèle de dose-réponse de Schnute–Jensen. Nous avons toutefois élargi l'analyse de Schnute–Jensen en décrivant les régions exactes d'inférence obtenues à partir des limites de la vraisemblance auxquelles nous avons attribué des niveaux de probabilité nominale. Nous fournissons également une méthode pour obtenir une région d'inférence pour la courbe prédite. Nous examinons la question de l'applicabilité pratique du modèle à l'aide de données sur la fécondité–longueur de deux espèces de sébaste (*Sebastes*). Nous faisons la preuve que la plus grande complexité de notre modèle est justifiée puisqu'aucun des modèles conventionnels de régression ne convient à nos trois ensembles de données. Nous utilisons de plus les régions d'inférence pour établir une distinction entre les relations fécondité-longueur pour le sébaste à dos épineux (*S. maliger*) provenant de deux endroits différents, mais nous sommes incapables d'établir une distinction avec l'une de ces relations pour le sébaste cuivré (*S. caurinus*).

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Traditional studies of rockfish (*Sebastes*) fecundity–size relationships have relied on linear or logarithmic models (e.g. Gunderson et al. 1980; Boehlert et al. 1982; Haldorson and Love 1990). Such simple models are often inadequate for these data, in that they fail to account for both curvilinearity and residual heterogeneity. In this paper, we describe a general method, based on the Schnute–Jensen (1986) dose–response model, for determining the relationship between fecundity and fish size or age. We demonstrate that five parameters may be necessary to describe such relationships completely. Two parameters pertain to an underlying linear trend, a third allows for curvilinearity, a fourth corrects heterogeneity in the residuals, and a fifth determines the residual variance. Although we emphasize rockfish size and fecundity, our methods are applicable to a wide range of relationships between a positive response y and a positive attribute x.

As reviewed by Seber and Wild (1989, section 2.8), the appropriate statistical model for describing a function $y(x)$ can be the subject of controversy. From one point of view, a suitable transform for y is chosen, and homogeneous residual variance is assumed during model selection and estimation. Alternatively, one can allow for heterogeneous variance and estimate a transform parameter for y simultaneously with other model parameters. The first method can lead to estimates that are deceptively precise, as uncertainty in the transform parameter is ignored (Carroll and Ruppert 1984).

The second method, which we adopt here, has been criticized for resulting in high correlations among the parameter estimates (Bickel and Doksum 1981). However, Box and Cox (1964, 1982) argued that high correlations are simply a mathematical artifact. Our approach involves self-scaling of the data and we demonstrate that the Bickel–Doksum criticism does not apply to our analysis. Furthermore, we obtain inference regions that are nearly elliptical, thus demonstrating that our choice of parameters leads to a model parametrically analogous to linear regression.

Although our model is based on Schnute and Jensen (1986), we extend their methodology in several directions. The analysis here depends primarily on likelihood inference, as espoused by Edwards (1972) and discussed in the context of sampling theory inference and Bayesian inference by Bates and Watts (1988). In particular, we describe exact inference regions obtained from likelihood contours, to which we associate nominal probability

levels. We also provide a straightforward method for obtaining inference bands for the predicted curve.

For completeness, we compare some of our results with corresponding results obtained from sampling theory. We find likelihood inference regions for a parameter pair to be consistent with parameter standard deviations and correlations obtained from the asymptotic covariance matrix. Our calculations are more exact than those of Schnute and Jensen (1986), as we compute the asymptotic covariance matrix numerically. By contrast, Schnute and Jensen (1986) use analytical formulas which are only approximate.

In the example below, we examine the issue of model adequacy as it relates to three fecundity–size data sets, obtained from two rockfish (*Sebastes*) species. We demonstrate that none of the traditional two-parameter regression models are appropriate for all three data sets. We also use inference bands to compare fecundity–size relationships among data sets. On this basis, we distinguish fecundity–size relationships for quillback rockfish (*S. maliger*) from two areas, but we are unable to distinguish one of these relationships from a similar relationship for copper rockfish (*S. caurinus*).

1. Model Description

In this section, we present two forms of the model, one for conceptual purposes and the other for numerical stability of the parameter estimates. Next, we describe the model's likelihood function and discuss methods for obtaining parameter inference regions. Finally, we present a method for computing inference bands for the predicted curve. To simplify the presentation, many of the equations are included in tables. We use square brackets to indicate these equations, so that (1.2) and [1.2] refer to the second equation in Section 1 and Table 1, respectively.

1A. The Model

The model is generally applicable to data (x_i,y_i), $i=1,..,n$, with $x_i>0$ and $y_i>0$. In our example, we let x_i represent the length of the ith fish and y_i represent a corresponding measure of fecundity. Conceptually, the model can be written

$$(1.1)\quad y_i^\gamma = a + bx_i^\alpha + \sigma\varepsilon_i,$$

where the ε_i, $i=1,..,n$, are independent normal random variables with mean 0 and variance 1. Model (1.1) involves five parameters: two regression parameters (a and b), two exponential parameters (α and γ), and a residual standard deviation (σ). Note that (1.1) is equivalent to a simple linear regression model when $\alpha=\gamma=1$.

To achieve numerical stability among parameter estimates, we use an alternative form of (1.1), proposed by Schnute and Jensen (1986). In this form, the model consists of the three equations

$$(1.2)\quad \xi_i = \frac{2x_i^\alpha - U^\alpha - u^\alpha}{U^\alpha - u^\alpha}$$

$$(1.3)\quad \eta_i = \frac{2y_i^\gamma - V^\gamma - v^\gamma}{V^\gamma - v^\gamma}$$

$$(1.4)\quad \eta_i = p + q\xi_i + \tau\varepsilon_i,$$

dependent on the parameters (p,q,α,γ,τ) and the prescribed quantities (u,U,v,V). Interrelationships among (a,b,σ) in (1.1) and (p,q,τ) in (1.2)–(1.4) are presented in Table 1. If α and γ are known, then (a,b,c) and (p,q,τ) are linearly related.

TABLE 1. Relationships among the parameters a, b, and σ in equation (1.1) and p, q, and τ in (1.2)–(1.4). Equations [1.1]–[1.3] determine (a,b,σ) from (p,q,τ), and [1.4]–[1.6] give (p,q,τ) from (a,b,σ).

[1.1] $a=\frac{1}{2}\left[V^\gamma+v^\gamma+(V^\gamma-v^\gamma)p-(V^\gamma-v^\gamma)\,(U^\alpha+u^\alpha)\,(U^\alpha-u^\alpha)^{-1}q\right]$

[1.2] $b=(V^\gamma-v^\gamma)\,(U^\alpha-u^\alpha)^{-1}q$

[1.3] $\sigma=\frac{1}{2}\,(V^\gamma-v^\gamma)\tau$

[1.4] $p=[2a+(U^\alpha+u^\alpha)\,b-(V^\gamma+v^\gamma)]\,(V^\gamma-v^\gamma)^{-1}$

[1.5] $q=(U^\alpha-u^\alpha)\,(V^\gamma-v^\gamma)^{-1}b$

[1.6] $\tau=2(V^\gamma-v^\gamma)^{-1}\sigma$

Conceptually, (1.2) and (1.3) represent transforms of x_i and of y_i in which (u,U,v,V) are fixed by the investigator prior to the analysis. In particular, u and U are chosen as typical low and high values of x_i, respectively. Similarly, v and V correspond to low and high values of y_i. With fixed (u,U,v,V) and specified parameters (p,q,α,γ,τ), a transformed value ξ_i can be obtained from x_i by (1.2), η_i can be obtained linearly from ξ_i by (1.4), and y_i can be obtained from η_i by the inverse of (1.3)

$$(1.5)\quad y_i = \left[\frac{V^\gamma+v^\gamma+(V^\gamma-v^\gamma)\eta_i}{2}\right]^{1/\gamma}.$$

Hence, the computational logic underlying (1.2)–(1.4) can be described as a series of links $x_i \to \xi_i \to \eta_i \to y_i$.

The model (1.2)–(1.4) is identical to the univariate form of the Schnute and Jensen (1986) dose–response model. Schnute and Jensen (1986) illustrate possible curve forms for the model and discuss limiting forms of the model equations as $\alpha \to 0$ and $\gamma \to 0$. Unlike (1.1), the model (1.2)–(1.4) still has meaning if $\alpha=0$ or $\gamma=0$. Similarly, all equations in this paper based on (1.2)–(1.4) have limiting forms as $\alpha \to 0$ and $\gamma \to 0$, although equations [1.1]–[1.6] based on (1.1) do not. Essentially, $\alpha=0$ and $\gamma=0$ correspond to logarithmic transforms of x_i and y_i, respectively. For example, as $\alpha \to 0$, (1.2) takes the limiting form

$$\xi_i = \frac{2\cdot\ln(x_i)-\ln(Uu)}{\ln(U/u)},$$

which is linearly related to $\gamma\ln(x_i)$.

The prescribed quantities (u,U,v,V) act to centre and scale the transform variables so that, for the most part, they lie in the range $(-1,1)$. Note that ξ_i in (1.2) goes from -1 to 1 as x_i goes from u to U. Similarly, η_i in (1.3) goes from -1 to 1 as y_i goes from v to V. Furthermore, because of this scaling, both p and q are near 1 in magnitude. Bickel and Doksum (1981) showed that correlations among a, b, and γ in (1.1) can be high. This problem is avoided with the parameters p, q, and γ for reasonable choices of (u,U,v,V), since the data are then scaled properly.

The link $\xi_i \to \eta_i$ from (1.4) involves the error $\tau\varepsilon_i$. To describe the likelihood function below, we also require a predicted $\tilde{\eta}_i$ from

$$(1.6)\quad \tilde{\eta}_i = p + q\xi_i.$$

Equation (1.6) leads to a prediction $\tilde{y}_i$ by (1.5). Thus, a series of predictive links is described by $x_i \to \xi_i \to \tilde{\eta}_i \to \tilde{y}_i$. Notice that y_i depends on the full parameter vector

TABLE 2. Likelihood functions and related quantities for model (1.2)–(1.4).

[2.1] $$L(\boldsymbol{\phi}) = \left(\frac{1}{2\pi\tau}\right)^n e^{-\frac{1}{2\tau^2}\sum_{i=1}^{n}(\eta_i - p - q\xi_i)^2} \prod_{i=1}^{n}\frac{\partial\eta_i}{\partial y_i}$$

[2.2] $$l_1(\boldsymbol{\phi}) = n\cdot\ln(n\tau^2) + \frac{1}{\tau^2}\sum_{i=1}^{n}(\eta_i - p - q\xi_i)^2 - n\cdot\ln\left(\frac{2\gamma Y^{\gamma-1}}{V^\gamma - v^\gamma}\right)^2 - n$$

[2.3] $$l_2(\boldsymbol{\theta}) = n\cdot\ln\left[\sum_{i=1}^{n}\left(\frac{y_i^\gamma - \tilde{y}_i^\gamma}{\gamma Y^{\gamma-1}}\right)^2\right]$$

[2.4] $$\tilde{y}_i = \frac{1}{2}[V^\gamma + v^\gamma + (V^\gamma - v^\gamma)(p + q\xi_i)]^{1/\gamma}$$

[2.5] $$Y = \left(\prod_{i=1}^{n} y_i\right)^{1/n}$$

[2.6] $$S(\boldsymbol{\theta}) = \sum_{i=1}^{n}[R_i(\boldsymbol{\theta})]^2$$

[2.7] $$R_i(\boldsymbol{\theta}) = \frac{y_i^\gamma - \tilde{y}_i^\gamma(\boldsymbol{\theta})}{\gamma Y^{\gamma-1}}$$

[2.8] $$\hat{\tau}^2(\boldsymbol{\theta}) = \frac{1}{n}\sum_{i=1}^{n}(\eta_i - p - q\xi_i)^2$$

$$\boldsymbol{\phi} = (p,q,\alpha,\gamma,\tau)$$

while $\tilde{y}_i$ depends only on

$$\boldsymbol{\theta} = (p,q,\alpha,\gamma).$$

A given $\boldsymbol{\theta}$ determines the predicted curve $\tilde{y}(x,\boldsymbol{\theta})$ for all values of x. The four components of $\boldsymbol{\theta}$ have conceptual significance as follows: p and q define an underlying linear trend, α allows for curvilinearity, and γ corrects for heterogeneous residual variance around the predicted curve. Our examples show that the roles of α and γ may be somewhat confounded, although both parameters are needed to insure model adequacy.

In this paper, we estimate all parameters $\boldsymbol{\phi}$ simultaneously. As mentioned in the introduction, an alternative approach might be first to make an ad hoc estimate of γ and then, with γ fixed at this value, to use standard nonlinear regression techniques for estimating (p,q,α,τ). Inferences from this approach ignore uncertainty in γ and may be too precise. Carroll and Ruppert (1984) found true asymptotic standard errors to be at most 1.2 times larger than those obtained with fixed γ. Their estimate may, however, be too low for the present context. In their simulation study, they used a much larger sample size and a narrower range of γ than is appropriate for our rockfish examples.

Our results support the philosophy of estimating all parameters simultaneously. This method at least simplifies the logistical problem of finding the correct model. Since ad hoc estimates for γ are usually obtained by trial and error, it is easier to avoid human intervention by letting the computer estimate all parameters at once.

1B. Model Likelihood

Likehood functions associated with model (1.2)–(1.4) are summarized in Table 2. The likelihood function $L(\boldsymbol{\phi})$ in [2.1] contains factors $\partial\eta_i/\partial y_i$ that convert the probability density for η_i to the density for y_i. Our analysis is based not on $L(\boldsymbol{\phi})$ directly, but on twice the negative log likelihood. In particular, we use the functions

$$(1.7)\quad l_1(\boldsymbol{\phi}) = -2\cdot\ln[L(\boldsymbol{\phi})] - K,$$

where $K = n + n\cdot\ln(4\pi^2/n)$ is a constant independent of $\boldsymbol{\phi}$, and

$$(1.8a)\quad l_2(\boldsymbol{\theta}) = \min_{\tau} l_1(\boldsymbol{\theta},\tau).$$

Expressions for $l_1(\boldsymbol{\phi})$ and $l_2(\boldsymbol{\theta})$ are given in equations [2.2] and [2.3], respectively. Equation [2.3] requires the prediction $\tilde{y}_i$ in [2.4] and the value Y, defined in [2.5] as the geometric mean of $y_1,..,y_n$. The minimum of $l_1(\boldsymbol{\phi})$ determines $\hat{\boldsymbol{\phi}}$, the maximum likelihood estimate for $\boldsymbol{\phi}$. Also, for a given $\boldsymbol{\theta}$, the estimate $\hat{\tau}(\boldsymbol{\theta})$ can be obtained by minimizing $l_1(\boldsymbol{\phi})$. The resulting value $\hat{\tau}(\boldsymbol{\theta})$ is given analytically in [2.8]. Thus, (1.8a) is equivalent to

$$(1.8b)\quad l_2(\boldsymbol{\theta}) = l_1(\boldsymbol{\theta},\hat{\tau}(\boldsymbol{\theta})).$$

Note that the maximum likelihood estimate $\hat{\boldsymbol{\theta}}$ of $\boldsymbol{\theta}$ can be obtained by minimizing either $l_1(\boldsymbol{\theta},\tau)$ or $l_2(\boldsymbol{\theta})$.

From [2.3] we obtain

$$(1.9)\quad l_2(\boldsymbol{\theta}) = n\cdot\ln[S(\boldsymbol{\theta})]$$

where $S(\boldsymbol{\theta})$ in [2.6] is the sum of squares of residuals R_i in [2.7]. The units of R_i are identical to those of y_i, and when $\gamma = 1$, R_i is the usual residual $y_i - \tilde{y}_i$. Equation (1.9) turns out to be useful in defining nominal probability levels for inference regions, as we discuss in section 1C.

1C. Confidence and Inference

In this section we develop methods for assessing uncertainty in the model's parameters from two points of view. First, we describe the classical asymptotic covariance matrix, based on the inverse Hessian of $l_1(\boldsymbol{\phi})$. Confidence intervals obtained by this method concur asymptotically with those obtained from sampling theory. Second, we describe likelihood inference regions to which we assign nominal probability levels.

The asymptotic covariance matrix may be obtained from the Hessian matrix $\mathbf{H}$ with elements

$$(1.10)\quad H_{ij} = \frac{1}{2}\cdot\left.\frac{\partial^2 l_1(\boldsymbol{\phi})}{\partial\phi_i\partial\phi_j}\right|_{\boldsymbol{\phi}=\hat{\boldsymbol{\phi}}}.$$

The factor 1/2 is included in (1.10) because of the factor 2 in (1.7). The asymptotic covariance matrix for $\hat{\boldsymbol{\phi}}$ is then given by

$$(1.11)\quad \boldsymbol{C} = \boldsymbol{H}^{-1}.$$

Although Schnute and Jensen (1986) provide approximate analytical formulas for $\boldsymbol{C}$,[1] in this paper we obtain $\mathbf{C}$ more exactly by computing numerical derivatives of $l_1(\boldsymbol{\phi})$. Once $\mathbf{C}$ is known, confidence regions for $\boldsymbol{\phi}$ can be constructed under the assumption that $\hat{\boldsymbol{\phi}}$ is multinormal. Since these regions are based on a quadratic approximation to $l_1(\boldsymbol{\phi})$, they have an ellipsoidal shape.

[1]For readers interested in estimating the covariance matrix as described in Schnute and Jensen (1986), we correct two typographical errors. Their equations (4.2) and (4.13) should read as follows:

$$(4.2)\quad F(\boldsymbol{X},y,\boldsymbol{\theta}) = \eta - \sum_{i=1}^{m} q_i\xi_i - p,$$

$$(4.13)\quad D(\boldsymbol{X}) = \sum_{k=1}^{N}\sum_{l=1}^{N} G_k(\boldsymbol{X},\tilde{y},\hat{\boldsymbol{\theta}})\, G_l(\boldsymbol{X},\tilde{y},\hat{\boldsymbol{\theta}})\, C_{kl}.$$

Likelihood methods recommended by Edwards (1972) provide an alternative to inferences based on **C**. From this point of view, likelihood contours

$$l_1(\boldsymbol{\phi}) = \text{constant}$$

define surfaces on which parameter values $\boldsymbol{\phi}$ have equal credibility. Higher values $l_1(\boldsymbol{\phi})$ correspond to parameters $\boldsymbol{\phi}$ with lower credibility. Thus, the function $l_1(\boldsymbol{\phi})$ is used to rank possible values of $\boldsymbol{\phi}$. The interior of a likelihood contour, termed an inference region (Bates and Watts 1988, p. 7), corresponds to $\boldsymbol{\phi}$ values at least as credible as those on the contour itself. Inference regions, analogous to confidence regions from sampling theory, reflect nonlinearities in the model and do not, in general, have an ellipsoidal shape.

It is sometimes convenient to treat one or more components of $\boldsymbol{\phi}$ as nuisance parameters. To achieve this, let $\boldsymbol{\phi} = (\boldsymbol{\phi}_1, \boldsymbol{\phi}_2)$, where $\boldsymbol{\phi}_1$ and $\boldsymbol{\phi}_2$ have k and m components, respectively, with $k+m=5$. Then, contours of

$$(1.12) \quad l_3(\boldsymbol{\phi}_1) = \min_{\boldsymbol{\phi}_2} l_1(\boldsymbol{\phi}_1, \boldsymbol{\phi}_2)$$

define inference regions for $\boldsymbol{\phi}_1$, with $\boldsymbol{\phi}_2$ considered nuisance parameters. Note that (1.8a) is a special case of (1.12) with $\boldsymbol{\phi}_1 = \boldsymbol{\theta}$ and $\boldsymbol{\phi}_2 = \tau$.

As defined, inference regions have no associated probability levels. However, based on arguments from classical sampling theory, it is possible to associate a nominal probability with the boundary of an inference region, i.e. a likelihood contour. One method involves the χ^2 likelihood ratio test, since minus twice a difference in log likelihoods is asymptotically χ^2 distributed. The true value of $\boldsymbol{\phi}_1$ in (1.12) can be considered to lie inside the contour.

$$(1.13) \quad l_3(\boldsymbol{\phi}_1) = l_3(\hat{\boldsymbol{\phi}}_1) + \chi^2_\nu(k)$$

with probability ν, where $\chi^2_\nu(k)$ is the ν-level for the χ^2 distribution with k degrees of freedom. Conceptually, the difference $l_3(\boldsymbol{\phi}_1) - l_3(\hat{\boldsymbol{\phi}}_1)$, i.e. the increase in $l_3(\boldsymbol{\phi}_1)$ above its minimum value, is scaled to probability units by the χ^2 distribution.

If $\boldsymbol{\phi}_1$ does not include τ, the F-distribution provides an alternative scaling method. In analogy with (1.13), the contour corresponding to nominal probability ν is then defined by

$$(1.14) \quad l_3(\boldsymbol{\phi}_1) = l_3(\hat{\boldsymbol{\phi}}_1) + n \cdot \ln\left[1 + \frac{k}{n-4} F_\nu(k, n-4)\right],$$

where $F_\nu(k, n-4)$ is the ν-level for the F-distribution with k and $n-4$ degrees of freedom. The conceptual basis for (1.14) is given in Appendix A. We show there that probability levels ν in (1.14) correspond formally to exact levels from linear regression theory.

We emphasize that the likelihood approach primarily involves ranking alternative parameter values. One can adopt either the χ^2 or the F scaling to attach ν-levels to likelihood contours. We prefer the F scaling because it is more conservative, i.e. it determines the larger region for a given ν. For large n, the two scales converge, since

$$(1.15) \quad n \cdot \ln\left[1 + \frac{k}{n-4} F_\nu(k, n-4)\right] \to \chi^2_\nu(k)$$

as $n \to \infty$.

1D. Model Prediction

Our analysis so far allows us to calculate the predicted curve

TABLE 3. Steps in the computation of $p_1(q,\alpha,\gamma)$ and $p_2(q,\alpha,\gamma)$ for given values of q, α, and γ. The data (x_i, y_i), $i = 1,..,n$, and (q,α,γ) are first used to compute A, B_i, C, D, and E in [3.1]–[3.5]. The quantities C, D, and E then determine p_1 and p_2 by [3.6]–[3.7].

[3.1] $A = (V^\gamma - v^\gamma)\,(2\gamma Y^{\gamma-1})^{-1}$

[3.2] $B_i = [(V^\gamma - v^\gamma)q\xi_i + V^\gamma + v^\gamma - 2y_i^\gamma]\,(2\gamma Y^{\gamma-1})^{-1}$

[3.3] $C = nA^2$

[3.4] $D = A \sum_{i=1}^{n} B_i$

[3.5] $E = \sum_{i=1}^{n} B_i^2 - S(\hat{\boldsymbol{\theta}}) \left[1 + \frac{4}{n-4} F_\nu(4, n-4)\right]$

[3.6] $p_1 = \dfrac{-D - \sqrt{D^2 - CE}}{C}$

[3.7] $p_2 = \dfrac{-D + \sqrt{D^2 - CE}}{C}$

$$(1.16) \quad \hat{y}(x) = \tilde{y}(x, \hat{\boldsymbol{\theta}}).$$

It remains to determine an inference band around (1.16) that includes the true curve $\tilde{y}(x)$ computed from the true but unknown $\boldsymbol{\theta}$. A lower bound for $\tilde{y}(x)$, associated with nominal probability ν, is defined by

$$(1.17) \quad y'(x) = \min_{\boldsymbol{\theta}} \tilde{y}(x, \boldsymbol{\theta}),$$

subject to the constraint

$$(1.18) \quad l_2(\boldsymbol{\theta}) \leq l_2(\hat{\boldsymbol{\theta}}) + n \cdot \ln\left[1 + \frac{4}{n-4} F_\nu(4, n-4)\right].$$

In other words, the lower bound at a value x is the lowest possible prediction for any $\boldsymbol{\theta}$ in the ν-level inference region. Similarly, an upper bound can be obtained from

$$(1.19) \quad y''(x) = \max_{\boldsymbol{\theta}} \tilde{y}(x, \boldsymbol{\theta}),$$

again subject to the constraint (1.18). For linear models, solutions to (1.17) and (1.19) result in the classical formulas given, e.g. by Bates and Watts (1988, p. 6) for a confidence band that include the true curve $\tilde{y}(x)$. In the present context, they allow us to obtain a ν-level inference band from a ν-level inference region for $\boldsymbol{\theta}$.

Numerical solutions to (1.17) and (1.19) depend on noting two features of the analysis. First, the minimum and maximum of $\tilde{y}(x, \boldsymbol{\theta})$ must occur on the boundary of the inference region. Thus, "$\leq$" in (1.18) can be replaced by "$=$" for the purposes of computation. Second, the constraint (1.18) determines a quadratic equation in the parameter p. This is apparent from [2.3], since $\tilde{y}_i^\gamma(\boldsymbol{\theta})$ depends linearly on p by [2.4]. Consequently, with q, α, and γ given, (1.18) determines two values of p. We refer to these as $p_1(q,\alpha,\gamma)$ and $p_2(q,\alpha,\gamma)$, with $p_1(q,\alpha,\gamma) \leq p_2(q,\alpha,\gamma)$. Thus, $y'(x)$ in (1.17) can be obtained from the unconstrained minimization

$$(1.20) \quad y'(x) = \min_{q,\alpha,\gamma} \tilde{y}(x, p_1(q,\alpha,\gamma), q, \alpha, \gamma).$$

Similarly, $y''(x)$ in (1.19) can be obtained from

$$(1.21) \quad y''(x) = \max_{q,\alpha,\gamma} \tilde{y}(x, p_2(q,\alpha,\gamma), q, \alpha, \gamma).$$

TABLE 4. Parameter transformations from (p,q,τ) to (p_*,q_*,τ_*), based on changing the prescribed quantities (u,U,v,V) to (u_*,U_*,v_*,V_*).

[4.1] $$p_* = \frac{V^\gamma + v^\gamma - V_*^\gamma - v_*^\gamma}{V_*^\gamma - v_*^\gamma} + \frac{V^\gamma - v^\gamma}{V_*^\gamma - v_*^\gamma} p + \frac{U^\alpha + u^\alpha - U_*^\alpha - u_*^\alpha}{U^\alpha - u^\alpha} \frac{V^\gamma - v^\gamma}{V_*^\gamma - v_*^\gamma} q$$

[4.2] $$q_* = \frac{U_*^\alpha - u_*^\alpha}{U^\alpha - u^\alpha} \frac{V^\gamma - v^\gamma}{V_*^\gamma - v_*^\gamma} q$$

[4.3] $$\tau_* = \frac{V^\gamma - v^\gamma}{V_*^\gamma - v_*^\gamma} \tau$$

Steps required in the calculation of p_1 and p_2 are listed in Table 3. A few technical points essential to implementing (1.20)–(1.21) are described in Appendix B.

We close this section with a final comment on the choice of (u,U,v,V). If these quantities are modified, they introduce a natural transformation on the parameters (p,q,τ). To be precise, Table 4 shows the transformation

(1.22a) $(p,q,\tau) \rightarrow (p_*,q_*,\tau_*)$

that results from the change in prescribed quantities

(1.22b) $(u,U,v,V) \rightarrow (u_*,U_*,v_*,V_*)$.

Based on [4.1]–[4.3], the parameters (a,b,σ) in Table 1 and the underlying model (1.1) are unchanged in the transformation (1.22). It follows that, given the transformations in Table 4, the likelihood functions (1.7)–(1.8) do not depend on the choice of (u,U,v,V). This means that all parameter inferences similarly honor these transformations. In particular, inference regions for (α,γ) and inference bands for the predicted curve are independent of (u,U,v,V).

2. Rockfish Example

Fecundity data used in the analysis were obtained from rockfish caught in commerical handline fisheries in the Strait of Georgia, British Columbia, Canada, in January 1986. Ovaries from a total of 130 quillback and five copper rockfish were collected near Campbell River and from 142 quillback and 16 copper rockfish near Nanaimo. These fishing areas are about 200 km apart. Female rockfish were measured for length to the nearest millimetre, and their ovaries were preserved for later examination. Details of sample handling are presented in Richards and Emmett (1988), along with counts of oocytes from at least four subsamples of each ovary. Fecundity (10^3 oocytes) was estimated as the product of ovary weight (mg) and the number of oocytes per unit weight (10^3 oocytes $\cdot$ mg^{-1}), derived from the subsample mean.

Analyses for quillback rockfish are presented by area. However, due to small sample size, data for copper rockfish have been combined over both areas. The three data sets are represented below by the abbreviations CO (copper rockfish), QB-CR (Campbell River quillback rockfish) and QB-NA (Nanaimo quillback rockfish). For all three data sets we chose

TABLE 5. Parameter estimates for the fecundity–length relationship, and minimum values of the likelihood function $l_1(\hat{\phi})$ for data sets CO, QB-CR, and QB-NA. There are nine cases for each data set, corresponding to all possible combinations of α and γ either unconstrained or constrained to 0 or 1. The analysis uses the quantities $(u,U) = (30, 40)$ (cm) and $(v,V) = (40, 400)$ with units 10^3 oocytes.

Case	Data set	$\hat{p}$	$\hat{q}$	$\hat{\alpha}$	$\hat{\gamma}$	$\hat{\tau}$	$l_1(\hat{\phi})$
1	CO	0.238	0.500	0	0	0.170	204.25
2	CO	0.211	0.537	1	0	0.208	212.70
3	CO	0.252	0.470	−0.570	0	0.163	202.48
4	CO	−0.062	0.421	0	1	0.275	227.82
5	CO	−0.082	0.469	1	1	0.242	222.44
6	CO	−0.109	0.504	2.325	1	0.226	219.57
7	CO	0.210	0.491	0	0.096	0.168	203.42
8	CO	0.125	0.514	1	0.298	0.185	206.94
9	CO	0.279	0.463	−0.810	−0.070	0.161	202.34
10	QB-CR	0.316	0.446	0	0	0.290	1697.27
11	QB-CR	0.328	0.443	1	0	0.296	1702.39
12	QB-CR	0.289	0.430	−2.306	0	0.284	1691.77
13	QB-CR	−0.076	0.524	0	1	0.396	1742.24
14	QB-CR	−0.061	0.530	1	1	0.395	1741.69
15	QB-CR	−0.062	0.529	0.922	1	0.395	1741.69
16	QB-CR	0.247	0.464	0	0.169	0.303	1695.01
17	QB-CR	0.244	0.467	1	0.210	0.311	1698.97
18	QB-CR	0.259	0.440	−2.062	0.078	0.291	1691.34
19	QB-NA	0.438	0.514	0	0	0.259	1948.76
20	QB-NA	0.467	0.478	1	0	0.266	1956.22
21	QB-NA	0.273	0.667	−5.098	0	0.241	1928.46
22	QB-NA	0.084	0.819	0	1	0.407	1922.95
23	QB-NA	0.125	0.774	1	1	0.408	1923.76
24	QB-NA	0.082	0.821	−0.038	1	0.407	1922.95
25	QB-NA	0.186	0.728	0	0.703	0.355	1918.59
26	QB-NA	0.200	0.705	1	0.771	0.370	1921.20
27	QB-NA	0.157	0.761	−2.088	0.556	0.328	1916.38

TABLE 6. Nominal probability levels, based on the F-distribution, for rejecting the given constrained model in favor of an unconstrained model. Probability levels are computed from (1.14) using the indicated choices for ϕ_1. Comparable values based on the χ^2-distribution in (1.13) are given in parentheses.

			Data set		
α	γ	ϕ_1	CO	QB-CR	QB-NA
0	0	(α,γ)	0.54 (0.62)	0.94 (0.95)	1.00 (1.00)
1	0	(α,γ)	0.98 (0.99)	1.00 (1.00)	1.00 (1.00)
*	0	(γ)	0.26 (0.29)	0.48 (0.49)	1.00 (1.00)
0	1	(α,γ)	1.00 (1.00)	1.00 (1.00)	0.96 (0.96)
1	1	(α,γ)	1.00 (1.00)	1.00 (1.00)	0.97 (0.98)
*	1	(γ)	1.00 (1.00)	1.00 (1.00)	0.99 (0.99)
0	*	(α)	0.64 (0.70)	0.94 (0.94)	0.86 (0.86)
1	*	(α)	0.94 (0.97)	0.99 (0.99)	0.97 (0.97)

$(u,U) = (30\text{ cm},\ 40\text{ cm})$ and $(v,V) = (40\times10^3$ oocytes, 400×10^3 oocytes). These values are near the lower and upper ranges of fish length and fecundity, respectively.

In order to obtain parameter estimates for the model (1.2)–(1.4), it is necessary to minimize $l_1(\boldsymbol{\phi})$ in [2.2] or to use the minimum of $l_2(\boldsymbol{\theta})$ in conjunction with the estimate $\hat{\tau}$ in [2.8]. We applied the simplex search method (Nelder and Mead 1965; Mittertreiner and Schnute 1985; Press et al. 1986) to $l_2(\boldsymbol{\theta})$. The resulting parameter estimates and minimum values

$$l_1(\hat{\boldsymbol{\theta}},\ \hat{\tau}\ (\hat{\boldsymbol{\theta}})) = l_2(\hat{\boldsymbol{\theta}})$$

are listed in Table 5 (cases 9, 18, and 27). Other cases in Table 5 illustrate how various constraints on α and γ affect parameter estimates and increase the value of $l_1(\hat{\boldsymbol{\phi}})$. These include the simple logarithmic ($\alpha=\gamma=0$) and linear ($\alpha=\gamma=1$) models. A total of nine combinations are considered for each data set, by constraining (α,γ) to (0,0), (0,1), (0,*), (1,0), (1,1), (1,*), (*,0), (*,1), and (*,*), where an asterisk indicates no constraint. Thus, the unconstrained model is denoted by (*,*).

The estimates $\hat{p}$ and $\hat{q}$ in Table 5 appear relatively robust to the constraints on (α,γ), reflecting an adequate choice (u,U,v,V). For the unconstrained model, there is a tendency for $\hat{\alpha}$ to be negative and $\hat{\gamma}$ to be positive or near zero. Generally, lower estimates $\hat{\tau}$ correspond to lower values $l_1(\hat{\boldsymbol{\phi}})$, especially for data set CO. This is not always true, however. Note from [2.8] that τ is dimensionless and measures the variance of $\eta_i-\tilde{\eta}_i$, i.e. the variance of error in the transformed variables. This differs from the variance of model residuals R_i in [2.6], which have the units of y_i.

The credibility of a constrained model, relative to the unconstrained model (1.2)–(1.4), can be evaluated by the nominal probability level ν obtained from (1.14). Table 6 lists the ν-values associated with comparisons between constrained and unconstrained models for the cases presented in Table 5. In each case, the constrained model fixes a choice of $\boldsymbol{\phi}_1$, and the difference $l_3(\boldsymbol{\phi}_1)-l_3(\hat{\boldsymbol{\phi}}_1)$ determines ν in (1.14). In sampling theory terms, the quantity $1-\nu$ defines the significance level for rejecting the constrained model in favor of the unconstrained model. We emphasize, however, that we consider the value of ν only as a scaling factor for the likelihood. From this point of view, the credibility of a constrained model decreases as the value of ν increases.

Note from Table 6 that no constraint on both (α,γ) appears to be acceptable ($\nu\leq0.95$) for all three data sets. For example, a logarithmic model ($\alpha=\gamma=0$) may be appropriate for data set CO ($\nu=0.54$), but it is somewhat marginal for QB-CR ($\nu=0.94$), and is clearly inappropriate for QB-NA ($\nu=1.00$). Similarly, a linear model ($\alpha=\gamma=1$) has consistent low credibility, with $\nu\geq0.97$. The only constrained model that is possibly credible with $\nu\leq0.95$ for all cases is $(\alpha,\gamma)=(0,*)$. However, the corresponding $\nu=0.94$ for data set QB-CR suggests that this possibility should be regarded with caution.

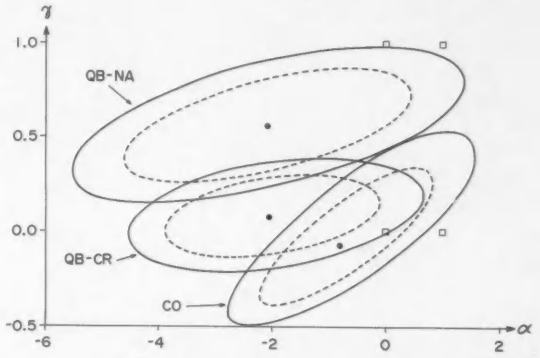

FIG. 1. Estimates (solid circles) and (α,γ) inference regions with $\nu=0.95$ (solid lines) and $\nu=0.80$ (broken lines) for cases 9, 18, and 27 in Table 5, corresponding to data sets CO, QB-CR, and QB-NA, respectively. Open squares indicate the points (0,0), (0,1), (1,0), and (1,1).

We have chosen to emphasize nominal probability levels obtained from the more conservative F-distribution. Scaling by the χ^2-distribution increases nominal probability levels by at most 1% for data sets QB-CR and QB-NA (Table 6). Differences are considerably greater, up to 8%, for data set CO. The larger differences reflect a sample size of only 21 fish for this data set, compared with sample sizes of over 100 fish for the other two data sets.

An alternative view of constraints on (α,γ) is provided by the (α,γ) inference regions in Fig. 1. The regions are computed from (1.14) with $\boldsymbol{\phi}_1=(\alpha,\gamma)$, where $\nu=0.80$ (broken line) and $\nu=0.95$ (solid line). The regions are all nearly elliptical in shape, suggesting that classical sampling theory approximations are valid. The four open squares correspond to the points (0,0), (0,1), (1,0), and (1,1). If constraints on (α,γ) corresponding to these points were credible, then the points would be included within the inference regions for the data set. The three squares (0,1), (1,0), and (1,1) are excluded from all regions. The fourth square at (0,0), is excluded from both regions for data set QB-NA and from the $\nu=0.80$ region for data set QB-CR. Similarly, the line $\gamma=0$ is excluded from both regions for data set QB-NA, although it passes through both regions for the other two data sets.

Table 7 lists standard deviations of the parameters (p,q,α,γ,τ) and their correlations, obtained numerically from (1.10)–(1.11). We include this table to compare sampling theory results with those of likelihood inference. The two methods, in fact, generally agree. Confidence intervals ($\nu=0.95$) for α and γ obtained from the standard deviations in Table 7 are consistent with the limits of the (α,γ) inference regions with $\nu=0.95$ in Fig. 1. Similarly, (α,γ) correlations correspond to slopes of the inference regions in Fig. 1 for each data set. In general, γ appears to be better determined than α, as standard deviations

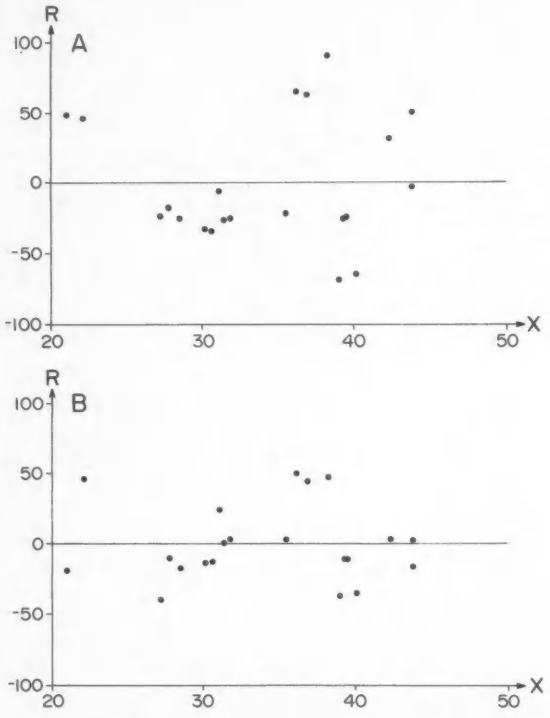

FIG. 2. Residuals (R, 10^3 oocytes) of the fecundity–length relationship as a function of length (x, cm) for data set CO. Panels A and B correspond to cases 5 and 9 in Table 5, respectively.

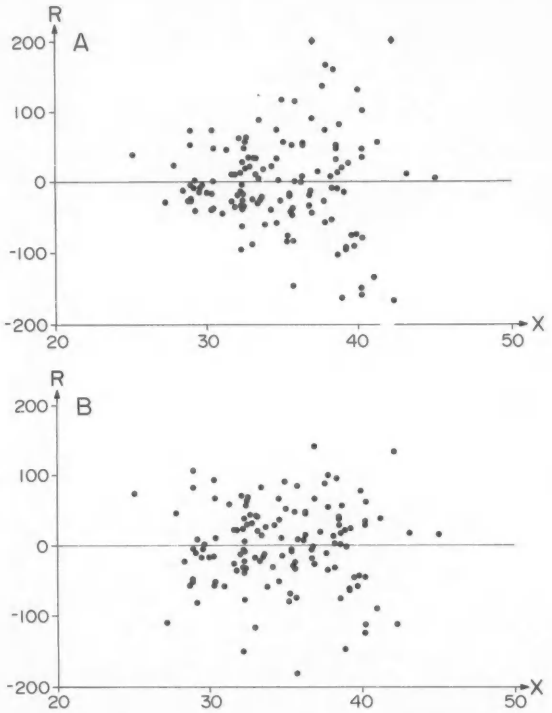

FIG. 3. Residuals (R, 10^3 oocytes) of the fecundity–length relationship as a function of length (x, cm) for data set QB-CR. Panels A and B correspond to cases 14 and 18 in Table 5, respectively. The diamonds in panel A indicate residual values greater than 200×10^3 oocytes.

TABLE 7. Parameter standard deviations and correlations from (1.10)–(1.11) for each data set. Corresponding parameter estimates are given in Table 5 by case 9 (CO), case 18 (QB-CR), and case 27 (QB-NA).

Parameters	CO	QB-CR	QB-NA
		Standard deviations	
p	0.077	0.053	0.065
q	0.039	0.037	0.066
α	0.740	1.024	1.397
γ	0.178	0.121	0.167
τ	0.025	0.021	0.034
		Correlations	
p,q	−0.448	−0.309	−0.835
p,α	−0.805	−0.137	0.262
p,γ	−0.876	−0.856	−0.482
p,τ	−0.162	−0.449	−0.397
q,α	0.758	0.466	−0.246
q,γ	0.474	0.419	0.380
q,τ	0.087	0.220	0.313
α,γ	0.837	0.369	0.594
α,τ	0.154	0.194	0.489
γ,τ	0.185	0.525	0.823

of α are as much as eight times larger than standard deviations of γ.

The controversial issue of model specification (Seber and Wild, section 2.8) centers on an appropriate transform for y, and whether or not the transform can be fixed a priori. This controversy may be misleading, in that it fails to consider the possibility of a fixed transform on x. In our analysis, the model is more robust to a constraint on γ than to a constraint on γ. Hence, if one of the transform parameters must be fixed, then α appears to be the better choice. In fact, from Fig. 1 the value $\alpha = -1$ seems appropriate for all three data sets. Then, model fit and variance homogeneity could be easily attained by estimating (p,q,γ). Of course, as we point out in section 1A, it is simpler and less equivocal to estimate all parameters simultaneously.

As discussed in section 1A, Bickel and Doksum (1981) argued that when γ is estimated simultaneously with other parameters, then $\hat{\gamma}$ can be highly correlated with other parameter estimates. The generally moderate correlations between parameter pairs (Table 7) indicate that estimates of the parameters in our model are reasonably uncoupled. Higher correlations for data set CO may reflect the smaller sample size. Note that the signs of the correlations between parameter pairs are consistent among data sets, with the exception of the (p,α) and (q,α) correlations.

An important step in final model selection is an examination of residual plots. For the model (1.2)–(1.4), residuals are defined by $R_i(\boldsymbol{\theta})$ in [2.7]. Figures 2 and 3 illustrate the main features of these plots for data sets CO and QB-CR, respectively. In both figures, the case with $\alpha = \gamma = 1$ is compared with

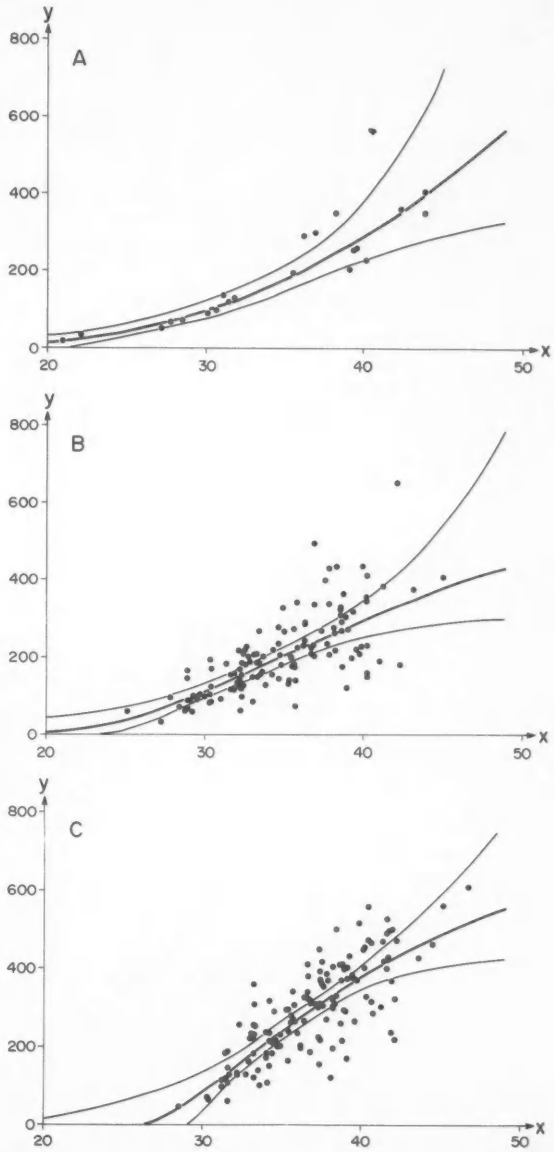

FIG. 4. Observed data points and the predicted curve (thick line) with the corresponding $\nu = 0.95$ inference band (thin lines) for the relationship between fecundity (y, 10^3 oocytes) and length (x, cm) for data sets (A) CO, (B) QB-CR, and (C) QB-NA. Parameter values correspond to cases 9, 18, and 27 in Table 5, respectively.

estimates from the unconstrained model. In Fig. 2A, two-thirds of the points R_i lie below the $R = 0$ axis, with positive residuals for low x and negative residuals in the mid-range of x. By contrast, the R_i have a narrower range and are more evenly distributed about the $R = 0$ axis in Fig. 2B. In Fig. 3A, the variance of the R_i appears to increase with fish length. The R_i are clearly more homogeneous in Fig. 3B. In general, the unconstrained model leads to an improvement in residual homogeneity and overall model fit.

The fecundity–length data, the predicted curves for the unconstrained model, and the corresponding inference bands with $\nu = 0.95$ are shown in Fig. 4. All three predicted curves (Figs. 4A–C) represent Schnute–Jensen (1986) curves of type 8, i.e. curves with an asymptotic fecundity y_∞. Estimates of y_∞, obtained by the methods of Schnute and Jensen (1986, equations (2.9)–(2.11), (A.1)–(A.2)), are 49.2×10^6 oocytes, 892×10^3 oocytes, and 971×10^3 oocytes for data sets CO, QB-CR, and QB-NA, respectively. These estimates all lie considerably above the range of observed data and have no biological significance.

Inference bands with $\nu = 0.95$ in Fig. 4 allow us to compare fecundity–length relationships among data sets. The bands for CO and QB-CR are not exclusive of each other; indeed, they overlap considerably. Thus, the relationships for these data sets cannot be distinguished. However, the inference band for QB-NA lies above the other two bands in the region where most of the data are located, indicating a greater fecundity for a given length.

In summary, we have shown that the extra complexity of the model (1.2)–(1.4) is justified over models involving linear or logarithmic constraints on α or γ. In particular, linear ($\alpha = \gamma = 1$) and logarithmic ($\alpha = \gamma = 0$) models, traditionally applied to rockfish fecundity–length relationships (e.g. Gunderson et al. 1980; Boehlert et al. 1982; Haldorson and Love 1990), cannot adequately describe all three data sets. We have also shown that likelihood inference methods provide a self-consistent framework for assessing uncertainty in model parameters and the predicted curve. Exact results from the method generally agree with those from sampling theory, based on the asymptotic covariance matrix.

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Appendix A

In this appendix, we show that the probability level ν in (1.14) corresponds to an exact probability if the model (1.2)–(1.4) is linear with four parameters. As $\boldsymbol{\phi}_1$ in (1.14) does not include τ, $\boldsymbol{\phi}_1$ can be considered part of $\boldsymbol{\theta}$. Thus, $\boldsymbol{\theta}=(\boldsymbol{\theta}_1,\boldsymbol{\theta}_2)$, where $\boldsymbol{\phi}_1=\boldsymbol{\theta}_1$. If the model were linear in $\boldsymbol{\theta}$, then classical least squares analysis would lead to a sum of squares of residuals $S(\boldsymbol{\theta})$. This could be minimized to obtain the estimate $\hat{\boldsymbol{\theta}}=(\hat{\boldsymbol{\theta}}_1, \hat{\boldsymbol{\theta}}_2)$ and for a prescribed $\boldsymbol{\theta}_1$, the estimate $\hat{\boldsymbol{\theta}}_2(\boldsymbol{\theta}_1)$. Furthermore, under the null hypothesis that $\boldsymbol{\theta}_1$ is correct

$$\text{(A.1)}\quad \frac{S(\boldsymbol{\theta}_1,\hat{\boldsymbol{\theta}}_2(\boldsymbol{\theta}_1))-S(\hat{\boldsymbol{\theta}}_1,\hat{\boldsymbol{\theta}}_2)}{S(\hat{\boldsymbol{\theta}}_1,\hat{\boldsymbol{\theta}}_2)}\,\frac{n-4}{k}\sim F(k,n-4),$$

where the expression on the left is a classical variance ratio.

This result can also be expressed in terms of the likelihood function, since in the regression context

$$\text{(A.2)}\quad l_2(\boldsymbol{\theta})=n\cdot\ln[S(\boldsymbol{\theta})]$$

is twice the negative log likelihood (exept for a constant) minimized over the residual variance τ^2. if we define

$$\text{(A.3)}\quad l_3(\boldsymbol{\theta}_1)=n\cdot\ln[S(\boldsymbol{\theta}_1,\hat{\boldsymbol{\theta}}_2(\boldsymbol{\theta}_1))],$$

then (A.1) can be restated in terms of l_3, rather than S. In particular, with probability ν, the true $\boldsymbol{\theta}_1$ lines inside the contour

$$\text{(A.4)}\quad \frac{e^{l_3(\boldsymbol{\theta}_1)/n}-e^{l_3(\hat{\boldsymbol{\theta}}_1)/n}}{e^{l_3(\hat{\boldsymbol{\theta}}_1)/n}}\,\frac{n-4}{k}=F_\nu(k,n-4).$$

After some algebra, (A.4) reduces to (1.14).

The analysis above is exact for linear regression models. Here, we simply make a formal identification between (A.2) and (1.9) and note that since $\boldsymbol{\phi}_1=\boldsymbol{\theta}_1$ and $\boldsymbol{\phi}_2=(\boldsymbol{\theta}_2,\tau)$, the definitions (1.12) and (A.3) are identical. Consequently, (1.14) is formally identical to classical analysis of variance based on (A.1).

Appendix B

In this appendix we include two technical notes on implementing the minimization (1.20) and maximization (1.21). First, it could happen during iteration that values (q,α,τ) are selected which do not lie on the constraint (1.18). In this case,

$$\text{(B.1)}\quad D^2-\text{CE}<0$$

in [3.6]–[3.7] and values of p_1 and p_2 are imaginary. We avoid this problem by setting $p_1=10$ and $p_2=-10$ when condition (B.1) occurs. This forces the iterative algorithm to search elsewhere for reasonable values (q,α,γ).

Second, the algorithm may attempt to evaluate $\tilde{y}(x,\boldsymbol{\theta})$ in a region where $\tilde{y}$ is not defined. If

$$\text{(B.2)}\quad \eta^*=\frac{v^\gamma+V^\gamma}{v^\gamma-V^\gamma},$$

then the prediction $\tilde{y}$ is not defined when

$$\text{(B.3a)}\quad \gamma>0 \text{ and } \eta<\eta^*$$

or

$$\text{(B.3b)}\quad \gamma<0 \text{ and } \eta>\eta^*.$$

To force the algorithm to avoid the regions (B.3), add a large penalty to the likelihood function, proportional to $(\eta-\eta^*)^2$, when conditions (B.3) occur.

Effects of Chlorpyrifos on the Diet and Growth of Larval Fathead Minnows, *Pimephales promelas*, in Littoral enclosures

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A series of 12 littoral enclosures constructed within a 2 ha, mesotrophic pond near Duluth, Minnesota were used to determine if sublethal concentrations of the insecticide chlorpyrifos [0,0,-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] could lead to changes in the diet and growth of fathead minnow larvae. Chlorpyrifos was added to the enclosures at nominal concentrations of 0.0, 0.5, 5.0, and 20.0 µg/L in a single application on June 16, 1986. Growth rates of larvae were significantly reduced in the treated enclosures during the 32-d study period. The most dramatic differences in the mean size of larvae from the four treatment groups were observed 15 d posttreatment. These differences corresponded to the most significant reductions in cladoceran, copepod, rotifer, and chironomid populations in the treated enclosures. These results indicate that toxicity to chlorpyrifos-sensitive invertebrate forage species forced dietary changes that led to reduced growth of native fathead minnow larvae in the treated enclosures.

On a utilisé une série de douze enclos littoraux construits dans un étang mésotrophique de 2 ha près de Duluth, dans l'État du Minnesota, pour déterminer si des concentrations subléthales de l'insecticide chlorpyrifos (thiophosphate de 0,0-diéthyle et de 0-(trichloro-3,5,6 pyridyle-2) amènent des variations du régime alimentaire et de la croissance de larves de tête-de-boule. Le chlorpyrifos a été ajouté à des concentrations nominales de 0,0, 0,5, 5,0 et 20,0 µg/l en une seule application effectuée le 16 juin 1986. Les taux de croissance des larves étaient nettement abaissés dans les enclos traités au cours de la période expérimentale de 32 jours. Les différences les plus marquées de la taille moyenne des larves des quatre groupes expérimentaux ont été observées 15 jours après le traitement. Ces différences correspondent aux plus importantes baisses des populations de cladocère, de copépode, de rotifère et de chironomidé dans les enclos traités. Les résultats révèlent que la toxicité du chlorpyrifos pour les invertébrés-proies qui y sont sensibles a forcé des modifications du régime alimentaire. Il en est résulté une croissance diminuée des larves du tête-de-boule indigène dans les enclos traités.

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The potential for pesticides in aquatic ecosystems to affect fish diet and subsequent growth is high, because toxicity to zooplankton and macroinvertebrates is often greater than for fish (Johnson and Finley 1980). Effects at lower trophic levels, such as phytoplankton (Boyle 1980; Day et al. 1987), periphyton (Giddings et al. 1984), epipelic algae (Stephenson et al. 1986), and zooplankton (Papst and Boyer 1980; de Noyelles et al. 1982; Kaushik et al. 1985) have been fairly clearly demonstrated. However, in mesocosm or whole ecosystem studies where the possibility of secondary effects on fishes were investigated, conclusively identifying secondary effects has been difficult (Boyle 1980; Eaton et al. 1985; Schindler et al. 1985; Mills et al. 1987).

Elimination or significant reduction in the density of important food items by a pesticide could reduce growth (e.g. O'Connell and Raymond 1970; Houde and Schekter 1981), increase vulnerability to predation (e.g. Cushing 1976), lead to starvation (e.g. Laurence 1977; Zale 1987), especially in the early life stages and ultimately reduce year-class strength (e.g. Miller et al. 1988). There have been numerous studies on the sensitivity of early life stages of fish to toxicants (for review see McKim 1977), but the linkages between a pesticide, the invertebrate forage base, and changes in fish diet are not clearly established.

We had an opportunity to investigate this possibility as part of a larger study on the effects of the mosquito larvicide, chlorpyrifos, on a pond ecosystem. This study investigated whether the addition of chlorpyrifos to littoral enclosures led to changes in the diet and growth of larval fathead minnows. Because chlorpyrifos is less toxic to larval fathead minnows than to many of the invertebrates utilized as food (Marshall and Roberts 1978; U.S. EPA 1986) we hypothesized that the addition of chlorpyrifos would reduce the abundance of invertebrates and cause diet changes that would result in reduced growth rates. The

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TABLE 1. Mean chlorpyrifos water concentrations (μg/L) measured in the treated enclosures (standard deviations in parentheses)[a].

Hours after application	Low	Medium	High
1	0.51(0.20)	6.29(1.05)	31.95(5.30)
12	0.30(0.04)	2.73(0.17)	11.64(2.11)
24	0.19(0.04)	1.36(0.19)	6.24(1.27)
96	0.11(0.00)	0.70(0.06)	2.90(0.67)
384	nd[b]	0.21(0.08)	0.56(0.10)
768	nd	0.05(0.02)	0.16(0.02)
Expected peak concentration	0.5	5.0	20.0
Loading rates (kg a.i./ha)[c]	0.003	0.028	0.123
Number of enclosures	2	4	4

[a]Adapted from Brazner et al. (1988).
[b]nd = Concentration below analytical detection limit (0.01 μg/L).
[c]Kilograms active ingredient per hectare.

results of this study are pertinent to the evaluation of the overall impact of pesticides on aquatic ecosystems.

Materials and Methods

Enclosure Description and Construction

The construction and design details for the 12 littoral enclosures used for this study are described elsewhere (Brazner et al. 1989). Briefly, the enclosures were rectangular (5 × 10 m), and bordered with plastic walls on three sides and natural shoreline on the fourth side. Each enclosure incorporated the natural sediments and the ambient densities of endemic pond biota at the time of enclosure. The average maximum depth of the enclosures was 1.1 m. The dominant aquatic plants were emergent cattails (*Typha angustifolia*), floating *Potamogeton natans*, and *Eutricularia* sp., and submergent *Chara* sp. The sediments were generally unconsolidated and highly organic.

The enclosures were constructed in three main steps. First, wooden walkways were installed around the perimeter of the area to be enclosed in the study pond. Next, walls of a 0.25-μm, woven-filament, polyolefin plastic (#888 H UV Clear Scrimweve®, Sto-Cote Products, Inc., Richmond, Illinois) and slatted-fencing were assembled to fit the dimensions of the areas to be enclosed. Finally, the walls were positioned on the walkways surrounding the areas to be enclosed, pushed into the sediments at the base of the walkways and fastened to the walkway support posts to complete the enclosure.

Experimental Design

The 12 enclosures were divided into two blocks with six enclosures each. Both blocks were located on the northwestern shoreline and were separated by about 30 m. The block locations were selected to maximize habitat similarity within and between blocks. Because it was important to have three concentrations of chlorpyrifos for other concurrent studies that were conducted, the use of two blocks required an unbalanced assignment of treatments (Brazner et al. 1988). Chlorpyrifos concentrations encompassed reported LC_{50} values for a wide variety of pond organisms (Marshall and Roberts 1978; U.S. EPA 1986) and the recommended application rates for mosquito control. Because toxicity data (Marshall and Roberts 1978; U.S. EPA 1986) and past experience with chlorpyrifos (Siefert et al. 1984, 1987) suggested that the low treatment and control enclosures would act as biological replicates for many species of pond biota and because we expected that many of the measurements from the medium and high treatment enclosures would have the largest variability associated with their means we decided to use two replicates for each of the control (0.0 μg/L) and low treatment (0.5 μg/L) groups and four for each of the medium (5.0 μg/L) and high treatment (20.0 μg/L) groups (Table 1). The replicates for the low and control treatment groups were assigned to the same block to avoid confounding possible block effects with pesticide effects.

Pesticide Application and Water Chemistry

Chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] emulsifiable concentrate Dursban® 2E (22.4% active ingredient) was applied on the morning of June 16, 1986. The appropriate amount of concentrate (depending on treatment level) was mixed thoroughly with 6 L of distilled water and applied over the entire enclosure water surface with a stainless steel hand sprayer and a 2.4-m wand to simulate a direct application for mosquito control. A single application was made to each of the 10 treatment enclosures. Composite water samples were collected at mid-depth from each enclosure 1 d prior to application and at 1, 2, 4, 8, and 12 h and 1, 2, 4, 8, 16, 32, 64, and 128 d after application. Water samples were analyzed for chlorpyrifos residue using gas liquid chromatography after liquid–liquid extraction with isooctane (2,2,4-trimethylpentane) (Brazner et al. 1988).

The chlorpyrifos water concentration 1 h after application (Table 1) in the low, medium, and high treatment enclosures were higher (2, 26, and 60%, respectively) than the target concentrations. However, replicates within treatment groups were very similar. Chlorpyrifos concentrations rapidly declined during the first 12 h, and then more gradually. They were below the detection limit (0.01 μg/1) by day 16, 64 and 128 in the low, medium and high treatment enclosures, respectively.

Fish Sampling and Analysis

Larval fathead minnows that were first observed in the enclosures on day −7 (6/09/86) were sampled on days −2, 7, 15, and 31 which closely coincided with zooplankton and macroinvertebrate sampling dates in each of the 12 enclosures. Fish were sampled by pulling a long handled dip net (500-μm mesh nitex, 25 × 40 cm opening) just under the surface of the water along the enclosure wall. Two 15-m tows were completed in each enclosure on each sample date. Sampled fish were anesthetized with ice to limit regurgitation and preserved in 10% formalin.

Relative larval densities were calculated for each sampling date. When sample sizes were adequate, 30 of these fish were weighed (wet weight) and measured (standard length), and 10 fish from each enclosure on each sample date were dissected for digestive tract contents analysis. Food items were identified to the lowest practical taxonomic group using keys by Edmondson (1959); Brooks (1959); Wilson (1959); Yeatman (1959); and Pennak (1978).

One way analysis of variance was used to test for initial (day −2 samples) and overall density (all samples) differences of larval fatheads among the individual enclosures and/or among the four treatment groups. Mean lengths and weights of larvae

on each of the four sampling dates were compared using a nested two-way analysis of variance with replicate enclosures from a specific treatment group nested within treatment (pesticide concentration) and block location. Where significant variance due to treatment was apparent, Tukey's HSD procedure was used to compare all pairs of treatment means. These analyses were completed using the general linear models procedure (SAS 1982).

Growth in length was described by the regression, $l_t = a + bt$, where l_t is the estimated length (mm) at day t relative to treatment and b is the daily growth rate (mm·d^{-1}). Growth in weight was determined from the exponential regression of wet weight (g), $W_t = W_o e^{Gt}$, where W_t is the estimated weight at day t relative to treatment, and G is the instantaneous daily growth coefficient. Percent daily weight gains were calculated as 100 $(e^G - 1)$. To test for homogeneity among the slopes of the growth regressions for the four treatment groups the general linear test approach described by Neter et al. (1983) was used. This was followed by the Tukey-Kramer MSD procedure for comparison of specific pairs of the regression slope coefficients (Sokal and Rohlf 1981). Differences were considered significant where $p \leq 0.05$ unless otherwise stated.

Initial analysis of the stomach content data was completed using the same form of two-way analysis of variance that was used for the length and weight comparisons. The means used for this analysis were derived from the $\log_{10}(x+1)$ transformed counts per fish of each of the major taxa observed in the samples (cladocerans, adult copepods, immature copepods, rotifers, protozoans, chironomids, and immature hydracarina) and for total counts per fish including all the major taxa on a given date. The number of individual forage types (species richness) per fish was also analyzed in this manner. In addition, the percent frequency of each of the major forage types was calculated from their geometric means so that Renkonen's index of proportional similarity (Renkonen 1938) could be derived for each of the three treatment groups relative to the control enclosures and for comparisons of the individual control enclosures to one another. Renkonen's index was calculated with the following formula:

$$\text{Percent similarity} = \Sigma \min(p_{1i}, p_{2i})$$

where p_i equals the proportion of species i in a particular sample and the summation is across all forage species. The frequency percentages were also used to calculate the linear index of food selection, L (Strauss 1979), to relate diet composition of fish to available forage. The index avoids most of the statistical and mathematical inadequacies of other food selection indices (Strauss 1979) and was used to determine whether preferred prey were eliminated following treatment. The index is calculated using the following formula:

$$L = r_i - p_i$$

where r_i is the relative quantity of any prey type in the digestive tract expressed as a percentage of the total gut contents and p_i is the relative quantity of the same prey type in microinvertebrate samples from the enclosures expressed as a percentage of the total number of microinvertebrates that were sampled.

Supplemental Trapping Study

A supplemental trapping study was performed to determine the number of native fish that were present in the enclosures, provide an estimate of each species density in each enclosure and reduce population numbers. Because of the potentially significant effect that fish could have on many of the system components measured for this study (Arumugam and Geddes 1986), greatly reducing or eliminating these populations was considered essential since the number of fish confined in each enclosure when the walls were set in place was not expected to be equal.

Four minnow traps (44.5 × 22.9 cm, 0.64 cm mesh) were placed in each enclosure for 24 to 48 h on 11 dates from day −11 through day 64. Sampled fish were identified, counted, and removed from the enclosures. The four minnow species found in the pond included fathead minnows, brook sticklebacks (*Culaea inconstant*), northern redbelly dace (*Chrosomus eos*), and mudminnows (*Umbra limi*). Sticklebacks were the most abundant species in most enclosures and the average number of adult fish trapped per enclosure typically ranged from 50 to 100. However, approximately 80% of all adult endemic fish had been trapped and removed from the enclosures by day 0, and virtully all adult minnows had been removed within 2 to 3 wk posttreatment. Based on the densities observed and the speed with which these populations were reduced, it seems unlikely that they had a significant impact on other components of the system.

Invertebrate Sampling and Analysis

Abundance of zooplankton and macroinvertebrates were estimated with funnel traps based on the design of Whiteside (1974) to supplement and assist in the interpretation of trends in diet and growth. Details associated with these methods are described in Brazner et al. (1988). The traps were set in the evening and removed the following morning. Samples were collected on days −3, 4, 16, and 32. Organisms were preserved in 10% formalin and identified to the lowest practical taxonomic level in the laboratory using keys by Balcer et al. (1984); Torke (1976); Edmondson (1959); Brooks (1959); Yeatman (1959); and Pennak (1978). Counts were $\log_{10}(x+1)$ transformed and analyzed with a nested two-way ANOVA.

Macroinvertebrates were collected on the same days as zooplankton with modified Hester-Dendy artificial substrate samplers (McDaniel 1974; Hester and Dendy 1962). Four samplers were suspended just above the bottom substrate in each enclosure 16 d prior to sampling. Samplers were removed with an 80-μm dipnet and preserved in 10% formalin. In the laboratory, organisms were washed from the samplers, collected in an 80-μm sieve and identified using keys by Wiederholm (1983); Pennak (1978); and Hilsenhoff (1975). Counts were analyzed in the same manner as the zooplankton data.

Results and Discussion

Fish Growth Rates

Growth rates of larval fathead minnows decreased significantly as chlorpyrifos concentration increased (Table 2). From day −2 to day 31 the larvae grew in length at mean rates ranging from 0.088 mm/d in the high treatment enclosures to 0.280 mm/d in the control enclosures. These growth rates were all significantly different from zero ($p \leq 0.01$) and the differences between the growth rates of the four treatment groups were all significant except for fish from the low and medium enclosures. However, the r^2 values (coefficients of determination) associated with these growth rates decreased with increasing concentration.

TABLE 2. Linear regressions describing growth in length and exponential regressions describing growth in weight of native, larval, fathead minnows sampled from day −2 to day 31 relative to chlorpyrifos treatment. For the length regressions, l = standard length (mm) t = day relative to treatment, b = growth rate (mm·d^{-1} and a is the y-axis intercept. For the weight regressions, W = wet weight (mg), t = day relative to treatment, G is the instantaneous growth coefficient, and W_0 = the theoretical weight (mg) at the start of the test.

Length

Treatment group	Number measured	Equation[a] $l = a + bt$	Standard error of b	r^2
Control	195	$x_1 = 10.2 + 0.28t$	0.008	69.6
Low	186	$y_1 = 10.5 + 0.16t$	0.010	49.7
Medium	439	$y_1 = 10.4 + 0.15t$	0.006	45.3
High	345	$z_1 = 9.9 + 0.09t$	0.007	19.7

Weight

Treatment group	Number measured	Equation[a] $W = W_0 e^{Gt}$	Standard error of G	r^2	Percent gain (%·d^{-1})
Control	195	$^xW = 5.30\ e^{0.080t}$	0.003	64.9	8.3
Low	186	$^yW = 6.41\ e^{0.049t}$	0.003	48.9	5.0
Medium	439	$^yW = 6.22\ e^{0.046t}$	0.002	46.0	4.7
High	345	$^zW = 5.04\ e^{0.031t}$	0.002	21.6	3.1

[a]Where superscripts preceding the length and weigh regression equations are different, the growth rates (b) and/or instantaneous growth coefficients (G) for those treatment groups differed significantly, $p \leq 0.05$.

Instantaneous growth coefficients ranged from 0.031 (3.1%·d^{-1}) in the high treatment enclosures to 0.080 (8.3%·d^{-1}) in the control enclosures (Table 2). As with the length regressions, all pairs of coefficients were significantly different from one another except those for the low and medium enclosures, and were significantly different from zero ($p<0.01$). Again, there was a decrease in the r^2 coefficients associated with these values as treatment concentration increased. This decrease in r^2 for both the length and the weight data indicates that as pesticide concentration increased, less of the change in length or weight of the fish could be explained by time alone, and implies that effects of the chlorpyrifos treatment contributed to the reduction in these values.

Initially (day −2), both mean length and weight were lowest (Fig. 1) for the control fish and about the same for the other three treatment groups; however, the differences were not statistically significant. By day 15, control fish were significantly larger than fish from the treated enclosures. This same pattern was still apparent among the treatment means on day 31, although the differences were less marked, indicating recovery had begun.

Based on stomach content analyses (Table 3), it appears that a reduction in several chlorpyrifos-sensitive invertebrate forage groups (Marshall and Roberts 1978; U.S. EPA 1986) in the treated enclosures contributed to the differences in fish growth (Tables 2, 3). In addition, the period of greatest reduction in invertebrate forage coincided with the timing of the greatest reductions in growth (Table 3). On day −2 there were no significant differences in the number of organisms consumed from any of the eight main forage groups, or in the total number of organisms consumed between any of the four treatment groups. Species richness and percent similarity of the diets (Table 3) were about the same at all treatment levels. However, by day 7, several significant differences were apparent. Mean

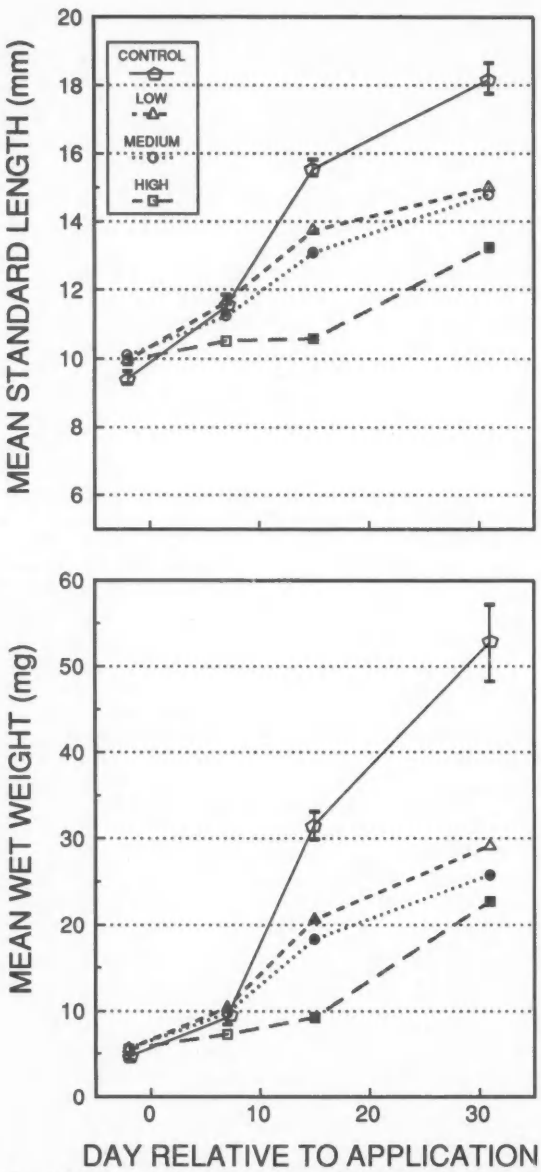

FIG. 1. Mean standard lengths and wet weights of larval fathead minnows sampled on days −2, 7, 15, and 31 relative to chlorpyrifos application (control means ±1 standard error, darkened treatment group symbols indicate significant differences from control, ANOVA, $p \geq 0.05$).

numbers of rotifers, cladocerans, protozoans, chironomids, immature hydracarina, mean total number of prey being eaten per fish, and mean species richness were greater in fish from the control enclosures than in some of the other treatment groups (Table 3). In addition, percent diet similarity between control fish and fish in the treated enclosures dropped sharply in contrast to the increase in similarity between the two control enclosures. On day 15, most of these differences were more pronounced, and an additional group, immature copepods, was found in

TABLE 3. Stomach content results given as geometric means (X) of organism numbers per larval fathead minnow sampled from the chlorpyrifos treatment and control enclosures and as frequency percentages (%); mean species richness and percent similarity of the diets is also included. Means for a given taxa and day with no letters in common in their superscripts are significantly different from each other, $p \leq 0.05$, pooled standard deviations were derived from the mean squared error term (MSE) used in the analysis of variance on the log transformations of these data, percent similarities based on comparison with mean control values, individual control enclosures compared with each other to provide the value in the control column, and n = the number of fish analyzed per mean. ^{r,b}Denotes where significant differences between replicate enclosures within a treatment group (r) or between blocks (b) were detected for a given forage group.

	Chlorpyrifos treatment								
	Control		Low		Medium		High		Pooled
Taxa	X	Percent	X	Percent	X	Percent	X	Percent	SD
Day 2									
Cladocerans	1.7	4.3	2.4	3.3	2.8	6.9	1.4	4.0	0.4
Adult copepods	1.1	2.8	1.1	1.5	1.3	3.2	1.2	3.4	0.2
Immature copepods	1.5	3.8	1.5	2.1	1.3	3.2	1.6	4.6	0.2
Rotifers	24.8	62.5	59.4	81.9	22.8	56.6	20.3	58.2	0.8
Protozoans	4.1	10.3	4.5	6.2	6.6	16.4	4.7	13.5	0.4
Chironomids	3.8	9.6	1.5	2.1	1.6	4.0	3.1	8.9	0.6
Immature Hydracarina	1.1	2.8	0	0	1.3	3.2	1.2	3.4	0.2
Other	1.6	4.0	2.1	2.9	2.6	6.5	1.4	4.0	0.8
Total^{r}	42.9		109.1		58.8		40.3		0.6
Species richness	9.2		6.0		7.4		7.7		0.8
Percent similarity	76.3		80.6		88.0		94.8		
n =	20		20		40		40		
Day 7									
Cladocerans^{r}	1.8^{a}	2.3	1.0^{a}	4.7	0^{b}	0	0^{b}	0	0.2
Adult copepods^{r,b}	1.9	2.4	1.4	6.5	2.4	9.6	2.3	13.7	0.7
Immature copepods^{b}	2.0	2.5	1.6	7.4	1.5	6.0	1.2	7.1	0.3
Rotifers^{r}	50.9^{a}	63.8	5.8^{ab}	27.0	9.7^{ab}	38.8	4.6^{bc}	27.4	1.0
Protozoans	16.7^{a}	20.9	6.2^{ab}	28.8	7.0^{ab}	28.0	5.2^{bc}	31.0	0.5
Chironomids	1.7^{a}	2.1	1.4^{b}	6.5	1.0^{c}	4.0	1.0^{c}	6.0	0.1
Immature Hydracarina^{r,b}	3.1^{a}	3.9	2.5^{ab}	11.6	1.7^{bc}	6.8	1.1^{c}	6.5	0.2
Other	1.7	2.1	1.6	7.4	1.7	6.8	1.4	8.3	0.3
Total^{b}	94.0^{a}		18.9^{b}		26.5^{b}		14.8^{b}		0.5
Species richness	12.0^{a}		6.6^{b}		6.3^{b}		4.7^{b}		1.1
Percent similarity	81.7		63.2		72.7		71.2		
n =	20		20		40		40		
Day 15									
Cladocerans^{r}	6.4^{a}	7.6	1.1^{o}	3.0	0^{b}	0	0^{b}	0	0.4
Adult copepods^{r,b}	1.6	1.9	1.1	3.0	1.4	6.0	1.2	16.9	0.2
Immature copepods^{r,b}	11.5^{a}	13.7	2.5^{b}	6.8	2.1^{b}	9.0	1.7^{b}	9.8	0.5
Rotifers^{r}	42.4^{a}	50.7	22.1^{a}	60.5	5.9^{b}	25.3	5.4^{b}	31.0	0.4
Protozoans	6.2^{a}	7.4	4.9	13.4	6.9	29.6	5.1	29.3	0.6
Chironomids	2.1^{a}	2.5	1.4^{b}	3.8	1.1^{b}	4.7	1.0^{b}	5.7	0.2
Immature Hydracarina^{b}	12.0^{a}	14.3	2.2^{bc}	6.0	4.7^{ab}	20.2	1.8^{c}	10.3	0.5
Other	1.5	1.8	1.2	3.3	1.2	5.2	1.2	6.9	0.2
Total^{b}	88.3^{a}		34.4^{ab}		22.8^{bc}		13.7^{bc}		0.5
Species richness	13.8^{a}		9.0^{b}		6.5^{b}		5.5^{b}		0.5
Percent similarity	85.5		80.1		62.2		64.7		
n =	20		20		40		40		
Day 31									
Cladocerans^{r}	2.0	7.2	6.0	0	0	0	0	0	0.5
Adult copepods;^{b}	1.4^{ab}	5.0	1.0^{a}	5.0	1.8^{ab}	13.6	2.8^{a}	19.4	0.3
Immature copepods	2.1^{a}	7.5	1.8	9.0	1.5	11.4	2.2	15.3	0.4
Rotifers^{r}	9.4^{a}	33.7	5.8^{a}	29.0	1.5^{b}	11.4	2.6^{b}	18.1	0.2
Protozoans	4.9	17.6	6.2	31.0	4.0	30.3	3.2	22.2	0.3
Chironomids	1.8	6.5	1.3	6.5	1.1	8.3	1.2^{b}	8.3	0.3
Immature Hydracarina^{b}	14.0^{a}	14.3	1.4^{b}	7.0	1.6^{b}	12.1	1.2^{b}	8.3	0.3
Other	2.3^{a}	8.2	1.3^{ab}	6.5	1.7^{ab}	12.9	1.2^{b}	8.3	0.2
Total^{b}	33.3^{a}		15.1^{b}		8.9^{c}		9.9^{c}		0.1
Species richness	7.8^{a}		7.0		3.8		4.4		2.1
Percent similarity	87.1		85.1		68.3		71.2		
n =	18		20		30		27		

significantly greater numbers in stomachs of control fish. By day 31, diet differences were less marked, but differences in the number of rotifers, hydracarina, and total number of prey consumed per fish in the control enclosures and the treated enclosures were still apparent.

This trend toward decreasing differences was due, in part, to the increasing reliance of all fish, especially those from the control enclosures, on algal and other detritus rather than individual prey items. Detritus typically accounted for between 25 and 50% of total stomach contents on day 31, whereas it was rarely detected in diets of fish sampled earlier in the study. This is a normal dietary change for fathead minnows as they mature beyond the postlarval life stage (Lord 1927; Isaak 1961). The decreasing diet differences by day 31 may have also been due to a partial recovery of the invertebrate forage communities in the treated enclosures. However, at no time did fish in the treated enclosures consume significantly more of a given prey type than fish in the control enclosures. In addition, fish from the control enclosures almost always had a greater number and variety of organisms in their stomach contents than fish from any of the treated enclosures.

The consistency and persistence of these diet differences indicates that foraging opportunities were better in the untreated enclosures and that larvae exhibited increased growth as a result of these opportunities. However, it is important to supplement stomach content data with data on the available forage during this same time period to substantiate this "better-forage/better-growth" hypothesis. It is possible that a direct sublethal effect on the fish led to foraging inefficiencies or reduced feeding by treated fish and contributed to the reduced growth. Chlorpyrifos is an acetylcholinesterase inhibitor (Marshall and Roberts 1978) and therefore, could lead to neurologically-induced behavioral anomalies. However, the high tolerances reported for fathead minnows to chlorpyrifos (U.S. EPA 1986) and the relatively short duration of greater than 1 μg/L concentrations, even in the high treatment enclosures (Table 1), make this seem unlikely.

Based on the zooplankton and macroinvertebrate samples, it appears more likely that the loss of chlorpyrifos-sensitive invertebrate forage species (Fig. 2) forced dietary changes that led to the observed reduction in fish growth (Fig. 1). Many of the forage taxa that decreased in the stomach contents after treatment (Table 3) also decreased in the invertebrate samples from the treated enclosures after treatment (Table 4). In addition, there were highly significant correlations between the total number of organisms available and both the total number

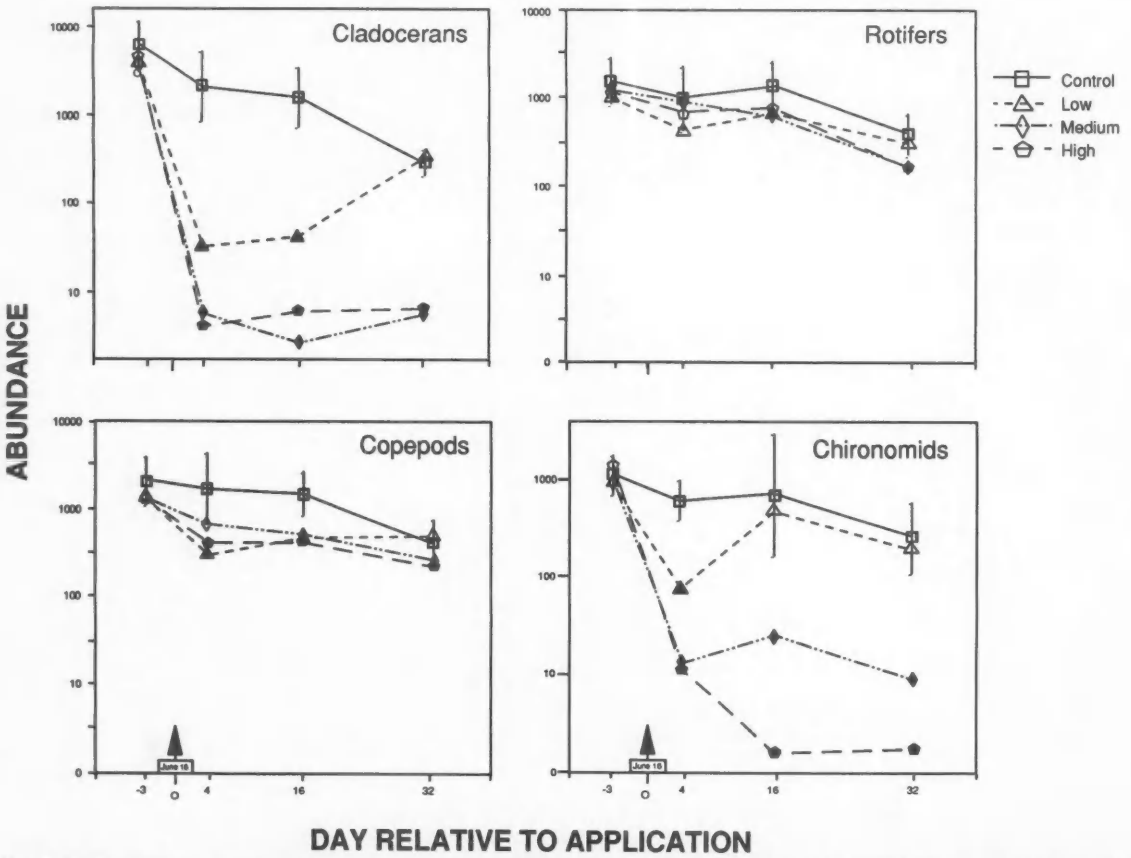

FIG. 2. Mean population responses of four important invertebrate forage types (cladocerans, chironomids, copepods, and roitfers) by treatment group (abundances are geometric mean number of organisms per square metre, except for chironomids which are number per artificial substrate sampler, control means ±2 standard errors, darkened treatment group symbols indicate significant differences from control, ANOVA, $p \leq 0.05$.

TABLE 4. Number of invertebrate taxa from each of the main invertebrate forage groups in the treated enclosures that were significantly reduced (ANOVA, $p \leq 0.05$) relative to the same taxa in the control enclosures (L = low treatment, M = medium treatment, H = high treatment).

	Day relative to application									
	4			16			32			Total taxa
Forage group	L	M	H	L	M	H	L	M	H	per group
Cladocera[a]	5	5	5	5	5	5	2	5	5	5
Copepoda[a]	1	2	2	1	1	3	0	1	2	7
Rotifera[a]	5	1	1	1	3	3	0	4	5	16
Ostracoda[a]	1	1	1	0	0	1	0	0	1	1
Chironomidae[b]	4	5	5	1	4	5	0	5	5	5
Protozoa[a]	0	0	0	0	0	0	0	0	0	1[c]
Hydracarina[b]	0	0	0	0	0	0	0	0	0	1[c]

[a]Based on data from funnel trap samples

[b]Based on data from artificial substrate samples

[c]≥6 species within this forage group were identified; all data were pooled prior to statistical analysis.

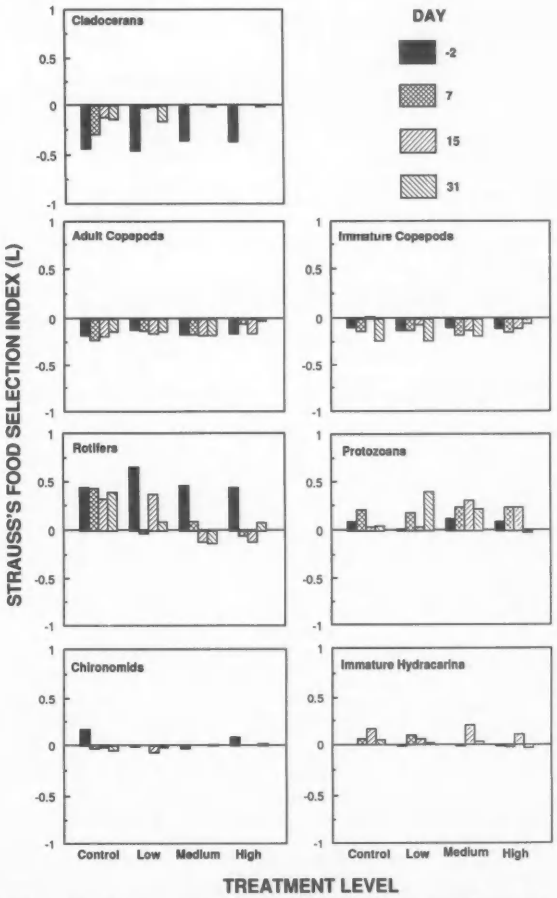

FIG. 3. Larval fathead minnow mean linear food selection indices "L" (Strauss 1979) for each of the major prey taxa, by sample day and treatment group.

of organisms in the fish gut ($r = 0.98$, $p < 0.01$) and the number of species in the fish guts ($r = 0.93$, $p < 0.01$). Further, fish growth rate was significantly correlated with these same two variables (total number of organisms per gut, $r = 0.79$, $p < 0.01$; number of species per gut, $r = 0.84$, $p < 0.01$). These correlations provide added support for the "better-forage/better-growth" hypothesis.

The results of the food selection calculations (Fig. 3) were generally neutral. Although there was a tendency towards positive selection of rotifers before treatment and protozoans after treatment, there was little indication that larval fathead minnows were selecting a particular forage type in strong preference over any other. These food selection results imply that if the growth differences observed were due to dietary changes forced by chlorpyrifos toxicity to forage organisms, diet changes were probably the result of a general decline in the forage base, rather than a specific reduction in a preferred prey type.

Growth differences observed between treatment groups may have been influenced by chance differences in the densities of larval or adult fish present in the enclosures, although fish densities for the 12 study enclosures were similar. There were no significant differences in the mean number of larval fathead minnows or total larval fish in any of the 12 enclosures or treatment groups sampled during the study. Densities typically ranged from 25–50 larvae·m^3 in each of the enclosures through day 31. Similarly, analysis of the native adult fish trapping results showed that there were no significant differences between the mean total number of native adult fish trapped from any of the four treatment groups.

The addition of chlorpyrifos to the littoral enclosures seems more likely to have resulted in direct toxicity to a variety of invertebrate species that were part of the larval fathead minnow forage base. As a result, diet changed and growth rates declined for the larvae, even at the lowest pesticide concentration. It is important to remember that the pesticide was applied in a single treatment early in the summer. Therefore, any effects noted represent a conservative estimate of response that might result from a more normal use pattern. Although chlorpyrifos was voluntarily withdrawn from direct application aquatic uses in November of 1985 by United States manufacturers, it is still used in a variety of agricultural applications. It is typically applied several times during the growing season and is recommended for as many as 22 applications per season (Miller 1985).

This study indicates that even low-level pesticide contamination in aquatic systems may result in indirect impacts on pesticide-insensitive organisms following direct toxic effects on organisms at lower trophic levels. This sort of effect may also be induced by other pollutants, such as metals, where the tolerance of invertebrate forage species to the pollutants is much lower than the tolerances of their fish predators (Eisler et al. 1979; Lemly and Dimmick 1982).

Miller et al. (1988) revealed that smaller larvae are relatively more vulnerable to starvation at any stage in development, not just around the "critical period" attributed to first feeding. Miller et al. (1988) also point out that the capture success of larval fish by a particular predator decreases as larval size increases and that larger larvae have fewer potentially successful predators than smaller larvae. This means that slower growing larvae will be more vulnerable to predation than fast growing larvae when all else is equal (Post and Prankevicius 1987).

Therefore, low levels of contaminants that induce slower growth in young-of-the-year fish through food chain effects or other means may eventually reduce survival and recruitment of these fish. Because of the potential ecological significance at the population, community, and even the ecosystem level we think that efforts should be made to detect whether these sorts of changes have occurred as part of the preregistration evaluation of risks associated with using a particular pesticide.

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Regulation of Pigment Sedimentation by Photo-Oxidation and Herbivore Grazing

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Reversed-phase high pressure liquid chromatography was used to show that algal carotenoid sedimentation is regulated by photo-oxidation and herbivore grazing in three unproductive, north-temperate lakes. Comparison of algal carotenoid and biovolume standing stocks revealed that carotenoid concentrations in epilimnetic waters were influenced by nonplanktonic inputs of pigments as well as phytoplankton community composition. Sediment traps did not record weekly events in the water column. Pigments that were incorporated in zooplankton feces and sank rapidly (e.g. alloxanthin and pheophorbide *a*) were overrepresented in trap material relative to epilimnetic pigment standing stocks. Differential decay of pigments in traps could not account for this observed bias. Chlorophyll mass–balance budgets corroborated the important influence of grazing by large-bodied zooplankton on pigment sedimentation. Interannual differences in pigment sedimentation were caused by changes in zooplankton community structure and the vertical zonation of phytoplankton communities. In the absence of fecal transportation, photo-oxidation destroyed carotenoids prior to their sedimentation from the photic zone. Therefore, sedimentary carotenoid composition is regulated by loss processes as well as changes in algal community composition.

La chromatographie liquide à haute performance en phase inversée a permis de montrer que la sédimentation des caroténoïdes des algues est réglée par la photooxydation et l'activité des herbivores dans trois lacs tempérés-nordiques improductifs. La comparaison des caroténoïdes des algues et de la biomasse vivante a révélé que les concentrations de caroténoïdes dans les eaux épilimnétiques étaient influencées par des apports non planctoniques de pigments ainsi que par la composition de la communauté phytoplanctonique. Les pièges sédimentaires n'ont pas enregistré d'événements hebdomadaires dans la colonne d'eau. Les pigments incorporés aux fèces de zooplancton et coulant rapidement au fond (p. ex., alloxantine et phéophorbide *a*) étaient surreprésentés dans les matériaux recueillis dans les pièges sédimentaires par rapport à la biomasse présente de pigments épilimnétiques. La différence d'altération des pigments dans les pièges ne peut expliquer ce biais observé. Le bilan de masse de la chlorophylle a corroboré l'influence importante de l'activité du zooplancton de grandes dimensions sur la sédimentation des pigments. Les différences interannuelles de sédimentation des pigments étaient attribuables à des changements de la structure de la communauté de zooplancton et à la zonation verticale des communautés phytoplanctoniques. En l'absence de transport de matières fécales, la photooxydation détruisait les caroténoïdes avant leur retrait par sédimentation de la zone photique. Par conséquent, la composition en caroténoïdes des sédiments est réglée par des processus de déperdition ainsi que par des modifications de la composition des communautés d'algues.

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Interpretation of the paleolimnological record requires clear understanding of factors that translate ecological events in the water column into sedimentary signals. Chlorophylls, carotenoids, and their derivatives are potentially versatile fossils (Brown 1969; Sanger 1988), yet studies coupling pelagic processes and pigment deposition are rare. In particular, determinants of carotenoid sedimentation are poorly documented. While algal community composition and epilimnetic carotenoid concentrations are correlated (Ridout and Morris 1985; Gieskes and Kraay 1986; Foy 1987), comparisons of standing crops, sediment-trap catch, and surface–sediment pigment composition show that selective degradation and deposition can occur (Repeta and Gagosian 1987; Hurley 1988).

Over a wide range of trophic status, pigment accumulation in lake sediments is correlated with primary production (Guilizzoni et al. 1983; Sanger 1988), both because of increased input and reduced degradation (Leavitt 1988). However, within a narrower range of nutrient loading, pigment production and sedimentation may be decoupled. In both marine and lacustrine systems, chlorophyllous pigments are rapidly photo-oxidized unless removed from the photic zone in feces of large cladocerans or copepods (Welschmeyer and Lorenzen 1985a, b; Carpenter et al. 1986, 1988). Grazing by zooplankton reduces carotenoid abundance, alters relative proportions, and creates novel derivatives (Repeta and Gagosian 1982; Barlow et al. 1988; Leavitt and Brown 1988), although carotenoids are less degraded than nonpigmented organic matter (Leavitt and Brown 1988). The importance of photo-oxidation in regulating carotenoid flux is unknown. The pigment content of detrital material is further modified by microbes and detritivores during settling through hypolimnetic waters (Leavitt and Carpenter 1990) and in surface deposits (Furlong and Carpenter 1988; Hurley 1988). The relative importance of degradative processes (photo-oxi-

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dation, grazing, hypolimnetic decay) has not yet been quantified for carotenoids.

We report the use of reversed-phase high pressure liquid chromatography (RP-HPLC) to compare carotenoid and chlorophyll standing stocks with algal community composition and pigment sedimentation in three north-temperate lakes. Construction of chlorophyll mass budgets and comparison with carotenoid loss processes and prior studies (Carpenter et al. 1986, 1988) allowed us to assign relative importance to photo-oxidation, hypolimnetic decay, and herbivore grazing as determinants of carotenoid sedimentation.

Materials and Methods

Peter, Paul, and Tuesday Lakes are small, seepage, kettle lakes located within 0.5 km of each other on the University of Notre Dame Environmental Research Center (Section 36, T45N R42W, Gogebic County, MI). The lakes have been the subjects of recent food web manipulations (Carpenter et al. 1987) and in 1986 were dominated by largemouth bass (*Micropterus salmoides*) and a *Daphnia–Holopedium* herbivore assemblage. Phytoplankton communities in each lake are described by Elser and Carpenter (1988) and Carpenter et al. (1987). Recent paleolimnological investigations (Kitchell and Kitchell 1980; Leavitt and Carpenter 1989; Leavitt et al. 1989) and studies of pigment fluxes (Carpenter et al. 1986, 1988; Leavitt and Carpenter 1990) make these lakes ideal for quantification of the mechanisms of carotenoid degradation. Our routine limnological methods are described elsewhere (Elser et al. 1986a, b; Carpenter et al. 1986, 1988; Leavitt et al. 1989) and will be recounted briefly.

Sampling

The study was restricted to the stratified period from 7 June to 3 September 1986 to minimize the influence of resuspended and allochthonous pigment sources. Lakes were sampled weekly from permanent stations over the greatest water depth. Solar radiation was measured continuously with a Belfort pyrheliometer.

Temperature and dissolved oxygen profiles were taken with a YSI meter and light penetration with a submersible quantum sensor and LiCor meter. Water samples for fluorometric pigment analysis and carbon fixation were obtained from Van Dorn casts to depths equivalent to 100, 50, 25, 10, 5, and 1% of surface irradiance. The three epilimnetic samples were also pooled and used for RP-HPLC pigment determination and phytoplankton enumeration. Additional water samples for HPLC analysis were taken at each trap depth. Zooplankton was collected by vertical hauls of a 75-μm mesh Nitex net.

Sediment traps consisted of three cylindrical collection chambers (5.08 cm inside diameter × 25 cm long) suspended at depth. Shallow traps were exposed to 1–3% surface irradiance, 1–3 mg $O_2 \cdot L^{-1}$, and 5–10°C. Deep traps were exposed to anoxic (<1 mg $O_2 \cdot L^{-1}$), aphotic waters and temperatures of 4–5°C at 9 m (Paul Lake), 10 m (Tuesday Lake), or 12 m (Peter Lake). Duplicate shallow and deep traps were collected biweekly and the contents of each trap pooled, mixed, and subsampled for pigment analysis. Trap contents were corrected for ambient seston levels at trap depth. Wall growth was negligible (<0.001% trap contents).

Pigment decay in the traps during trapping periods was corrected using coefficients determined for these lakes (Leavitt and Carpenter 1990). Assuming degradation follows first-order kinetics and daily pigment inputs to traps are constant during deployment, corrected sedimentation (S, μmol·m^{-2}) is given by

$$(1)\quad S = t(pk[1-\exp^{tk}]^{-1}),$$

where t = duration of trap deployment (d), p = observed trap catch (μmol pigment·m^{-2}) and k = hypolimnetic decay constant (d^{-1}) (Leavitt and Carpenter 1990).

Limnological Analyses

Carbon fixation, primary production, and routine chlorophyll concentrations were estimated using the methods of Carpenter et al. (1986, 1988). Fluorometric chlorophyll *a* values were converted to RP-HPLC equivalents based on empirical ratios from 13 pooled epilimnetic samples and seven shallow-trap-depth samples in each lake. Pigments from pooled epilimnetic samples, sediment traps, and photo-oxidation experiments were quantified using RP-HPLC (Leavitt et al. 1989).

Pigments were isolated, identified, and calibrated using the procedures of Leavitt and Brown (1988) and Leavitt et al. (1989). Analysis was restricted to chlorophyll *a*, *b*, and *c*, pheophytin *a*, pheophorbide *a*, and the carotenoids alloxanthin (cryptophytes), fucoxanthin (Chrysophyta), and lutein-zeaxanthin (not separated; chlorophytes and cyanophytes). Chlorophyll from ^{14}C-labelling experiments was analyzed by normal-phase HPLC (Carpenter et al. 1986, 1988) because labelled, nonpigmented material coelutes with chlorophyll *a* (chl *a*) on RP-HPLC chromatograms.

Phytoplankton was preserved with Lugol's iodine, settled, and counted with an inverted scope. Volume and biovolume estimates were calculated using the procedures of Elser et al. (1986a). Pigment–volume ratios were calculated directly from pooled epilimnetic samples (see below). Preserved zooplankton was counted and animal sizes (exclusive of spines and setae) determined with a dissecting microscope. Crustacean and rotifer masses were estimated from lengths using the regression equations of Downing and Rigler (1984, table 7.2) and Peters and Downing (1984).

Chlorophyll Budgets and Carotenoid Degradation

Mass budgets were developed for chl *a* and its major derivatives for both the mixed layer and photic zone in each lake using procedures modified from Carpenter et al. (1986, 1988). Mass budgets (in units of μmol·m^{-2}) consisted of pigment inputs due to chlorophyll production (P) and nonplanktonic inputs (N) balanced against pigment outputs due to the change in pigment standing stock in the water column (D), pigment photo-oxidation (X), sedimentation (S), and decay during trap deployment (H). All elements of the budget were measured directly except nonplanktonic inputs, which were calculated by difference

$$(2)\quad N_{tot} = D_{tot} - P_{chl} + X_{cdp} + S_{tot} + H_{tot},$$

where the *tot* subscript refers to the sum of chl *a* and its derivatives, the *chl* subscript refers to chl *a*, and the subscript *cdp* refers to chlorophyll degradation products (pheophytin *a*, pheophorbide *a*). The tautomer chl *a*′ was not quantified because it always accounted for <10% of chlorophyllous pigments. We assumed all chl *a* was protected from photo-oxidation in living cells (Carpenter et al. 1986). Therefore, no term appears for photodegradation of chl *a*.

Production

Chlorophyll production was estimated from depth-integrated determinations of carbon fixation (PPR, μmol $C \cdot m^{-2} \cdot wk^{-1}$) and the ratio of algal carbon to chl *a* (*C:Chl*, mmol C (μmol chl *a*)$^{-1}$)

$$(3) \quad P_{chl} = PPR/(C:Chl),$$

where *C* and *Chl* are initial values determined in monthly chlorophyll-labelling experiments at depths corresponding to 50 and 10% of surface irradiance (Redalge and Laws 1981; Carpenter et al. 1986). To determine *C:Chl*, total incorporation of ^{14}C into algae (*A**, $dpm \cdot L^{-1}$) was measured, and carbon fixation (*F*, $\mu mol \cdot L^{-1}$) calculated. The specific activity of HPLC-purified chlorophyll (*R**, $dpm \cdot \mu mol^{-1}$) was then used to calculate initial algal carbon (Ci, $\mu mol \cdot L^{-1}$) and initial carbon to chlorophyll ratios

$$(4) \quad Ci = (A^*/R^*) - F$$

$$(5) \quad C:Chl = Ci/Chl$$

Photo-oxidation experiments

Photodegradation rates of carotenoids and chlorophylls were measured as a function of light intensity using a procedure modified from Carpenter et al. (1986). Pigment-rich water was obtained from deep-trap depth in each lake, pooled and incubated in transparent 1-L Nalgene bottles at a depth equivalent to 50% surface irradiance. Each bottle contained 10 μM DCMU and 100 μM NaN_3 to poison autotrophs and heterotrophs. NaOH was not added (Carpenter et al. 1986) because preliminary experiments showed it promoted degradation of base-sensitive carotenoids (e.g. fucoxanthin). Minimally, triplicate light bottles and a foil-covered dark control were retrieved every 3 h from 700 to 1600 h. Photo-oxidation rate constants (*K*, $m^{-2} \cdot E^{-1}$) were calculated for each pigment by linear regression of $\ln(P_l - P_d)$ versus *I*, where P_l and P_d are light and dark-bottle pigment concentrations and *I* is photon flux ($E \cdot m^{-2}$) at incubation depth.

Daily photo-oxidation of pheophytin *a* and pheophorbide *a* was calculated from rate constants, RP-HPLC ratios of pheopigment:chl *a* (pheo:chl, $\mu mol \cdot \mu mol^{-1}$) and interpolated profiles of chl and irradiance for each lake. Daily photo-oxidation of each pigment, *j*, (X_j, $\mu mol \cdot m^{-2} \cdot d^{-1}$) in a given depth is

$$(6) \quad X_j = (\text{pheo:chl})\ chl_f\ [1 - \exp^{-Kjl}]$$

where chl_f is fluorometric chl *a* as RP-HPLC equivalents ($\mu mol \cdot m^{-3}$) and *Kj* is the photo-oxidation constant ($m^{-2} \cdot E^{-1}$). Total photodegradation of chlorophyll derivatives was obtained by integrating over depth and time and summing for pheophytin *a* and pheophorbide *a* (Carpenter et al. 1986).

Grazing rates

Pigment ingestion by herbivores was estimated using published regression models (Peters and Downing 1984) and observations from mesocosm experiments (Bergquist et al. 1985; Bergquist and Carpenter 1986). Weekly ingestion rates were calculated by dividing both phytoplankton and zooplankton into 11 size-classes based on cell or colony volume and animal size, respectively. For each of the 121 possible combinations, maximum edible particle size (Burns 1968) and mean filtering rate (equation (2), Peters and Downing 1984) were calculated for herbivorous zooplankton.

Edibility of algae was determined in two ways. First, if maximum edible particle size was greater than the radius (*r*) of the algal cell or colony, then the alga was considered edible. Mean algal radius of each size-class was determined from volume estimates ($r = [3v(4\pi)^{-1}]^{1/3}$). Greatest axial linear dimensions (GALD) were not used because the ingestion regression (Burns 1968) assumes cells are spherical and underestimates edibility of non-spherical cells. This procedure excluded a high proportion of the algae from herbivore diets. A more inclusive estimate resulted when all cells <30 μm in GALD were considered edible. Algae smaller than 30 μm GALD respond differently than larger algae to increases in grazer concentration in these lakes (Bergquist et al. 1985; Bergquist and Carpenter 1986; Elser 1987). Cells and colonies smaller than 30 μm GALD are consistently grazed, while responses are more variable for larger taxa. While the 30 μm cutoff is somewhat arbitrary, model output was not sensitive to increases in the edibility parameter (30 to 50 μm).

In either case, the ingestion rate of each inedible size-class was set to zero, while the ingestion rate of edible algae was determined using equation (5) of Peters and Downing (1984). Total grazing was calculated by summing ingestion across zooplankton size-classes and time.

Pigment ingestion was calculated by running both forms of the model for all phytoplankton (chl *a*, pheophytin *a*, pheophorbide *a*) or, in the case of the carotenoids, for only the appropriate taxa. Volume of algae ingested was converted to the appropriate pigment ($\mu mol \cdot m^{-2}$) using pigment:volume ratios empirically determined from epilimnetic samples. By using both exclusive (regression-based) and inclusive (mesocosm-based) estimates of ingestion, we bracketed the true rate of pigment consumption.

Mean algal sinking rates were calculated under two extreme scenarios to estimate the importance of pigment degradation relative to particle settling. Maximum possible sinking rates were calculated assuming spherical cells with no form resistance ($\phi = 1.0$) and a completely static (nonturbulent), unstratified water column. Estimates of minimum sinking rate assumed maximum form resistance ($\phi = 4.0$) and a turbulent water column (Reynolds 1984). Rates were expressed as sinking half-lives (time for half the population to sink from the photic zone, in days). In both cases, radius was calculated from mean cell or colony volume (see above), algal density was assumed to be 1075 $kg \cdot m^{-3}$ and water temperature 20°C.

Results

Standing Crops and Sedimentation

Correlations between changes in epilimnetic carotenoid standing stocks and their representative algal populations spanned a wide range (Fig. 1). In Paul and Tuesday Lakes, fucoxanthin concentrations were significantly correlated with total biovolume of Chrysophyta ($r = 0.85$, $P < 0.0001$), but not in Peter Lake where these algae were rare. Alternately, lutein–zeaxanthin standing stock was unrelated to changes in total biovolume of Chlorophyta and Cyanophyta ($P > 0.1$), although changes in the relative abundance of lutein–zeaxanthin and that of chlorophytes and cyanophytes was significant in Paul Lake ($r = 0.67$, $P < 0.01$). Alloxanthin concentration was weakly correlated with cryptophyte biovolume in Paul Lake ($r = 0.50$, $P = 0.07$), but not in the other lakes.

Chlorophyll concentrations (not shown) followed similar patterns with chl *c* levels and Chrysophyta biovolume correlated in Paul ($r = 0.80$, $P < 0.001$) and Tuesday Lakes ($r = 0.60$,

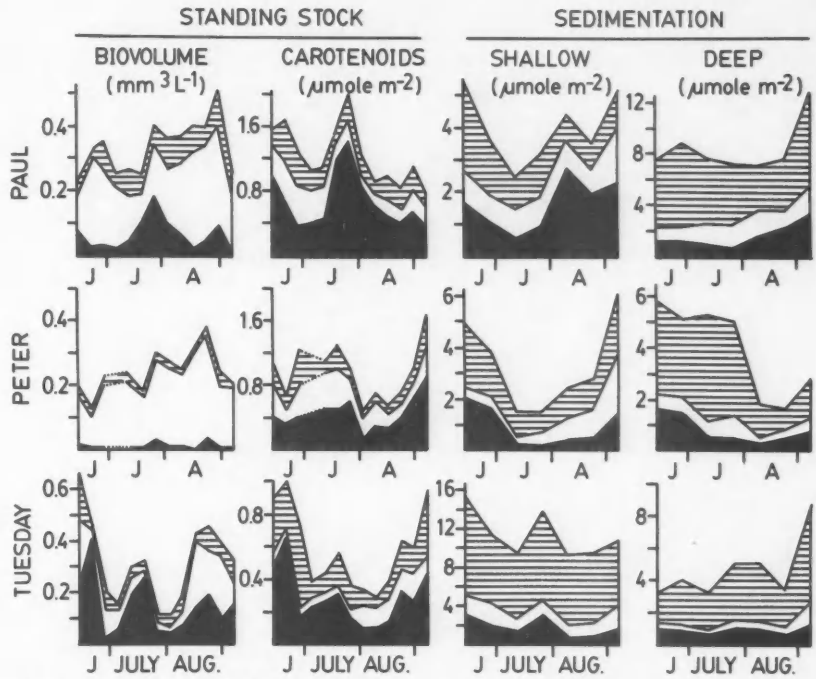

FIG. 1. Epilimnetic algal biovolume ($mm^3 \cdot L^{-1}$) and carotenoid standing stocks ($\mu mol \cdot m^{-2}$), and shallow and deep sediment trap carotenoid catches ($\mu mol \cdot m^{-2}$) in Paul, Peter, and Tuesday Lakes in 1986. Black = Chrysophyta and fucoxanthin, white = green and blue-green algae and lutein–zeaxanthin, hatched = cryptophytes and alloxanthin. Dashed line = missing sample.

$P<0.05$), but not Peter Lake. Chlorophyll *b* concentration was correlated to chlorophyte biovolume in Paul ($r=0.69$, $P<0.01$) and Tuesday Lakes ($r=0.65$, $P<0.01$).

Relative abundance of indicator carotenoids was different from the relative importance of the algal populations that produced them (Fig. 2). In Paul and Peter Lakes, fucoxanthin and chl *c* (not shown) overestimate the abundance of the Chrysophyta at the expense of the chlorophyte–cyanophyte category. Relative abundance of carotenoids in Tuesday Lake is similar to that of their representative algal groups.

Comparison of carotenoid standing crops with pigment catch in shallow and deep sediment traps revealed two patterns (Fig. 1). First, the amount of alloxanthin in the shallow traps greatly overestimated either biovolume or carotenoid standing-stock estimates of cryptophyte abundance in the water column. This effect was most marked in Tuesday Lake, although large increases in the relative abundance of alloxanthin were seen in all deep-trap samples (Fig. 3). In contrast, chl *c* (not shown) remained the most important native chlorophyll in all samples. Among the *a*-phorbins, pheophorbide *a* increased with depth at the expense of both chl *a* and pheophytin *a* (Fig. 3). Second, the fine-scale changes in epilimnetic carotenoid concentrations were not well preserved in the traps. Individual events were phase-shifted to later dates and poorly resolved in shallow traps, while deep-trap catch appeared completely decoupled from pelagic events. Loss of resolution of pelagic events was expected because of both differences in sampling intensity (weekly versus biweekly) and the time required for algal sedimentation (Reynolds et al. 1982).

Trap ratios (deep/shallow catch) showed distinct differences in patterns of sedimentation between lakes (Fig. 4). Ratios were greater than unity for most pigments in Paul Lake throughout the study, whereas the opposite pattern occurred in Tuesday Lake. An intermediate situation occurred in Peter Lake where deep-trap catch usually exceeded shallow early in the study, but the situation was reversed by mid-July. Timing of the reversal was similar to that for the sharp reduction in carotenoid standing stock in Peter Lake (Fig. 1).

Degradation Experiments

Photo-oxidation constants differed among lakes, with most rapid pigment loss in transparent Peter Lake (Fig. 5). This contrasts with 1984, when phorbin losses were slowest in Peter Lake (Carpenter et al. 1986). Among carotenoids, alloxanthin was most susceptible to bleaching in Paul and Peter Lakes, while lutein–zeaxanthin was greater in Tuesday Lake. Photodegradation constants of *a*-phorbins were broadly similar to those of carotenoids and values obtained in 1984. Pheophorbide *a* loss rates were lower than those of chl *a* and pheophytin *a*, while photo-oxidation of chl *a* was usually intermediate to that of chl *c* (greatest) and chl *b* (least).

For most pigments, hypolimnetic decay was greatest in Peter Lake and least in Tuesday Lake, where losses were often undetectable (Fig. 6). Fucoxanthin was the most rapidly degraded carotenoid in traps in Paul and Tuesday Lakes, while lutein–zeaxanthin required the largest correction in Peter Lake. Alloxanthin decomposition was intermediate in all lakes. Chlorophyll *a* was the most labile *a*-phorbin in Peter and Paul Lakes, in contrast to Tuesday Lake (pheophytin *a*).

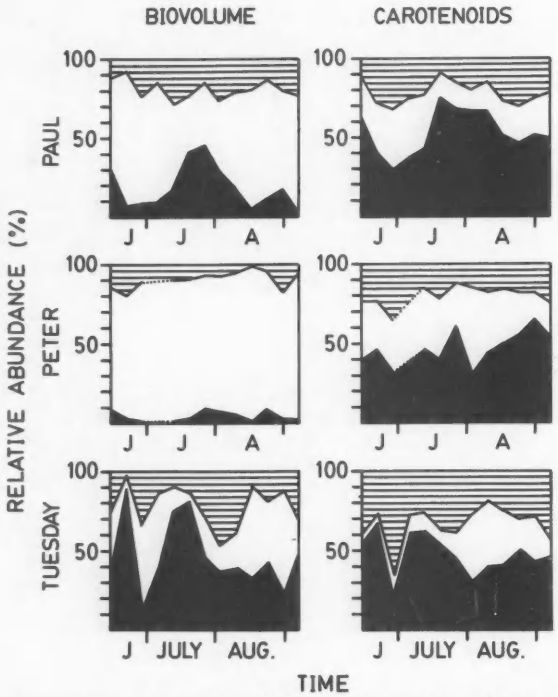

FIG. 2. Relative (%) abundance of algal groups in epilimnetic waters in 1986 based on algal biovolume or carotenoid standing stocks. Symbol colors as in Fig. 1.

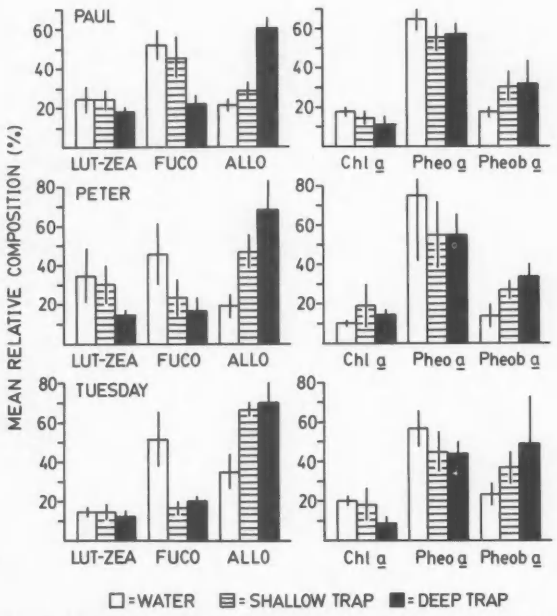

FIG. 3. Mean annual relative (%) pigment composition in epilimnetic waters (open), shallow traps (hatched), and deep traps (solid) in 1986. Lut/zea = Lutein–zeaxanthin, Fuco = fucoxanthin, allo = alloxanthin, Pheo *a* = pheophytin *a*, pheob *a* = pheophorbide *a*. Error bars = one standard error of the mean. $N = 13$ for water samples and seven for traps.

Estimates of pigment consumption by herbivores showed that cryptophytes were generally most easily ingested, regardless of model assumptions (Fig. 7). The exclusive model, which estimated edibility as a function of cell radius and herbivore length (Fig. 7a), suggested that cryptophytes alone were affected by grazing zooplankton. Ingestion rates of *a*-phorbins were most similar to lutein–zeaxanthin estimates, indicating that most chl *a* was associated with the chlorophyte–cyanophyte category. Similar results were obtained when inclusive assumptions of edibility (all cells <30 μm) were made (Fig. 7b). While all estimates of ingestion increased greater than two-fold under the inclusive model, fucoxanthin was the most dramatically affected (>5× increase), indicating that the Chrysophyta were particularly sensitive to assumptions of edibility based on cell morphology.

Chlorophyll Budgets

Carbon:chlorophyll ratios differed among lakes and between depths within each lake (Fig. 8). Generally, ratios increased with both water clarity (Peter>Paul>Tuesday) and light levels at incubation depth (50% surface irradiance >10%). Exceptions to this pattern occured in the first two determinations in Paul Lake where *C:Chl* values for 50% light were the lowest recorded in any experiment. These low ratios, combined with moderate chl standing stock (Fig. 3) and carbon fixation, translated into greatly increased rates of chlorophyll production relative to Peter and Tuesday Lakes (Fig. 9).

Mass–balance budgets were constructed for both photic (Table 1) and epilimnetic zones (Table 2) by combining estimates of chlorophyll production and *a*-phorbin loss. As in 1984–85 (Carpenter et al. 1986, 1988), chl *a* production was insufficient to account for summed loss processes in Peter or Tuesday Lakes. Nonplanktonic inputs were at least as important as production in both systems. While production in Paul Lake exceeded output by ca. 38%, this difference was within the range of error produced in previous budgets of these lakes (Carpenter et al. 1986, 1988). Sedimentation of *a*-phorbins was the most important output in stained Paul and Tuesday Lakes, while photo-oxidation was the principal means of phorbin degradation in all lakes and the primary output in Peter Lake. Pigment decay in sediment traps was less important than that due to photo-oxidation in all lakes and both budgets (Tables 1, 2).

Discussion

Biovolume and Carotenoids

Individual carotenoids differed in the accuracy with which they monitor algal populations (Fig. 1). Fucoxanthin was well correlated with the *Mallomonas caudata*-dominated Chrysophyta in Paul and Tuesday Lakes ($r = 0.85$, $P < 0.0001$), but not in Peter Lake where the Chrysophyta were rare and redistribution of fucoxanthin-rich littoral sediments (Leavitt and Carpenter 1989) was an important pigment input (Tables 1, 2). Poor correlations ($r < 0.50$, $P > 0.05$) between alloxanthin concentration and cryptophyte (*Cryptomonas ovata*, *Rhodomonas minuta*) biovolume resulted from relatively constant populations of these taxa. Limited range of the independent variable (i.e. biovolume) can produce poor correlations even if biovolume and alloxanthin concentration are directly related (Box et al. 1978). Alloxanthin has no

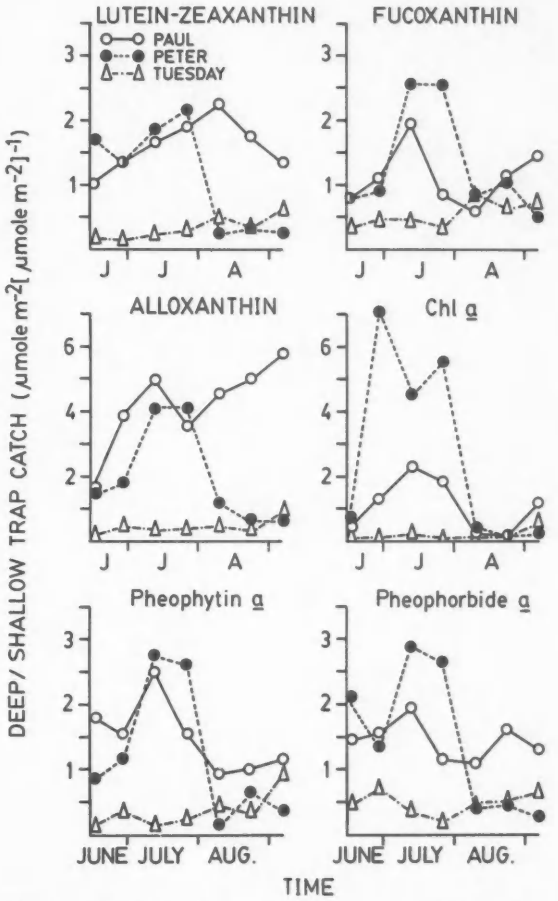

FIG. 4. Ratio of deep to shallow sediment–trap catch ($\mu mol \cdot m^{-2}(\mu mol \cdot m^{-2})^{-1}$) in 1986 for Paul (open circle), Peter (solid circle), and Tuesday Lakes (open triangle).

terrestrial component and is in low concentration in littoral sediments (Leavitt and Carpenter 1989). Because cellular pigment contents vary greatly among taxa (Goodwin 1980), species replacements in the cyanophyte–chlorophyte group (Carpenter et al. 1987) contribute to weak correlations ($P>0.1$) between biovolume and lutein–zeaxanthin concentration. Other studies have found weak correlations between carotenoids and algal abundance, even in systems lacking nonplanktonic inputs (e.g. Ridout and Morris 1985; Gieskes and Kraay 1986). Our results show that estimates of the relative abundance of phytoplankton groups depend on whether carotenoids or biovolumes are used (e.g. Fig. 2).

Pigment–biovolume relations may also be affected by ambient light levels (Fig. 8). Generally, carbon-specific chl *a* concentrations were inversely related to light intensity, as noted in other studies of chlorophyll content (Goodwin 1980). Similar variation may occur with carotenoid concentrations. Because algal biovolume and carotenoid standing stocks are influenced by different factors and measure different aspects of phytoplankton populations, the most interesting comparisons are between changes in epilimnetic carotenoid composition and those recorded in sediment traps. These are the relationships

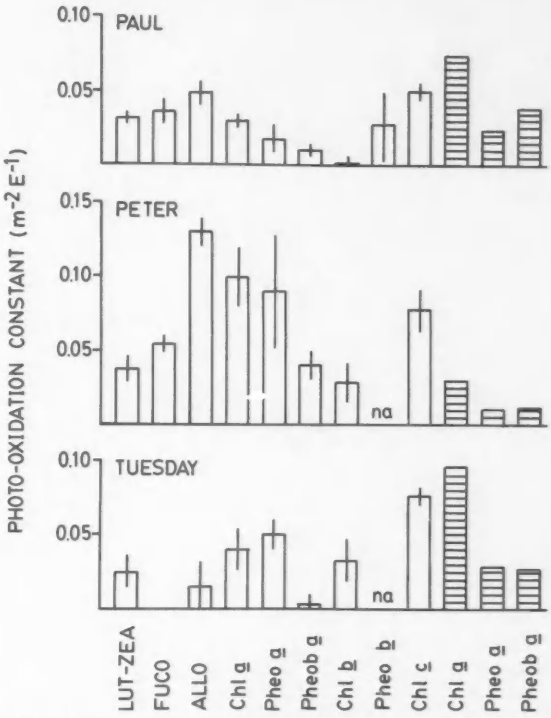

FIG. 5. Photo-oxidation constants ($m^{-2} \cdot E^{-1}$) for carotenoids and chlorophyllous pigments for 1986 and 1984 (hatched, from Carpenter et al. 1986). Error bars and pigment abbreviations as in Fig. 3. Na = pigment absent.

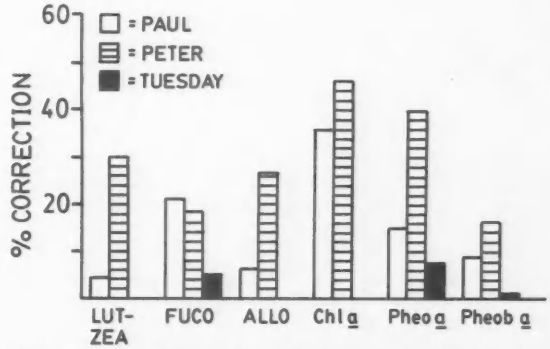

FIG. 6. Correction (percent pigment sedimented) required for decay in deep sediment traps. Some Tuesday Lake corrections are negligible. Pigment abbreviations as in Fig. 3. White = Paul Lake, hatched = Peter Lake, and black = Tuesday Lake.

that affect the utility of carotenoid sedimentation for paleolimnology.

Carotenoid Sedimentation and Degradation

Sediment traps did not record either the fine-scale temporal dynamics or relative quantities of epilimnetic carotenoids (Fig. 1). Sediment-trap material had a higher proportion of alloxanthin and pheophorbide *a* than did epilimnetic seston

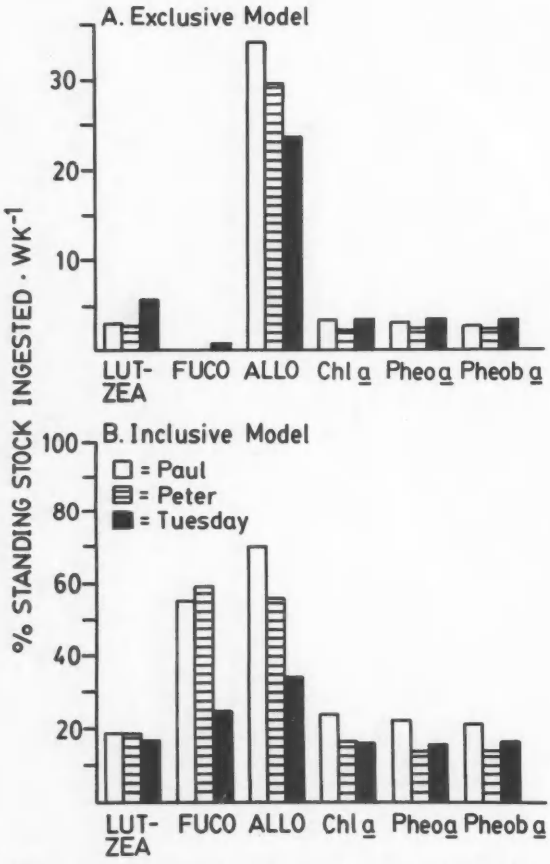

FIG. 7. Percent of carotenoid standing stock consumed per week by herbivorous zooplankton assuming (a) algal ingestion is proportional to cell radius (calculated from volume estimates) and herbivore length and (b) all cells <30 μm in diameter are edible. Lake symbols as in Fig. 6.

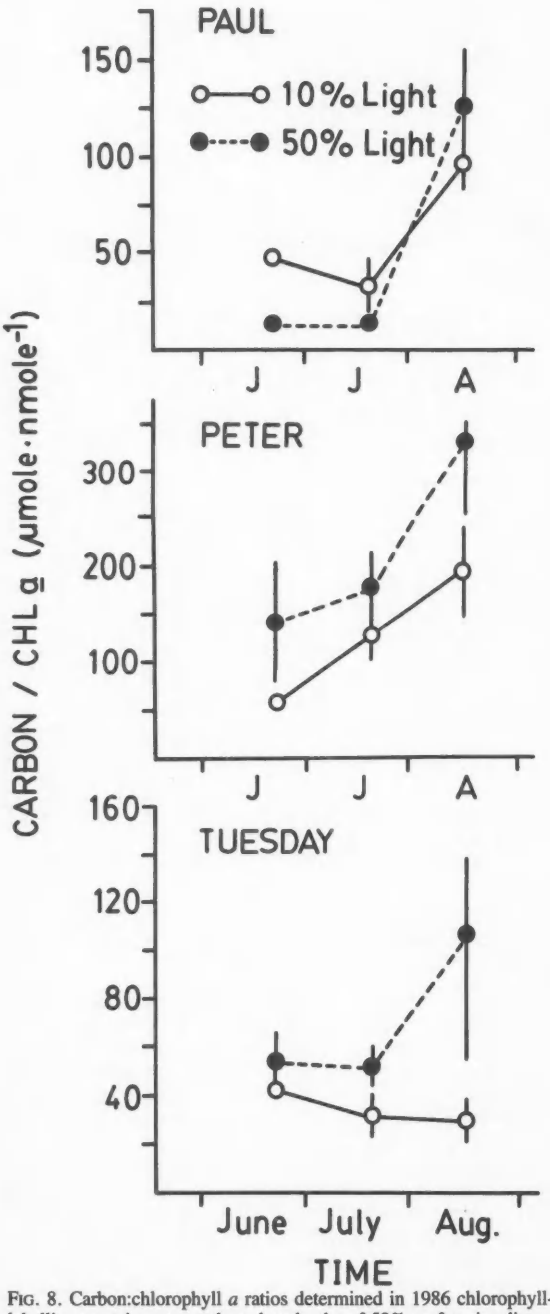

FIG. 8. Carbon:chlorophyll *a* ratios determined in 1986 chlorophyll-labelling experiments conducted at depths of 50% surface irradiance (●– – –●) and 10% irradiance (○——○)). Error bars as in Fig. 3. $N = 3$.

(Fig. 3). Such overrepresentation of alloxanthin in deepwater sediments is a common observation in both freshwater and marine studies (Repeta and Gagosian 1987; Hurley 1988; Leavitt et al. 1989).

Overrepresentation of alloxanthin and pheophorbide *a* in trap material relative to epilimnetic seston cannot result from low rates of photo-oxidation (Fig. 5). Alloxanthin decay constants were greater than those of other carotenoids in both Peter and Paul Lakes, yet overrepresentation occurred in all three lakes. In fact, photodegradation of alloxanthin may be even greater than indicated solely by decay kinetics. Based on biomass-weighted mean cell GALD (G_w) or mean volume-estimated cell diameter (D), cryptophytes ($G_W = 21.1$ μm, $D = 11.3$ μm) should sink more slowly than either the Chrysophyta ($G_w = 49.4$ μm, $D = 36.8$ μm) or members of the chlorophyte–cyanophyte category ($G_w = 107.6$ μm, $D = 22.2$ μm) (Table 3). Even under assumptions that maximize algal sinking rate (spherical cell, no water column turbulence), bleaching of alloxanthin can reduce standing crops to undetectable levels in less time than required for sedimentation of pigments (Table 3). Thus, losses of alloxanthin should exceed those of other carotenoids because of both rapid bleaching and longer residence time in the photic zone. Because all lakes had similar cryptophyte species compositions, interlake differences in alloxanthin decay constants likely result from the presence of humic acids, which absorb light at the same wavelength as carotenoids (Wetzel 1983).

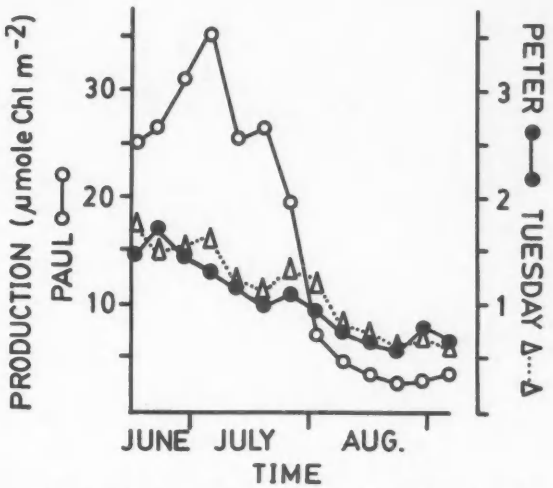

FIG. 9. Chlorophyll *a* production ($\mu mol \cdot m^{-2}$) in 1986 in Paul, Peter, and Tuesday Lakes. Lake symbols as in Fig. 4.

TABLE 1. Photic zone budget of chl *a* in Paul, Peter, and Tuesday Lakes in 1986 ($\mu mol \cdot m^{-2}$). Calculated from equation (2).

	Paul Lake	Peter Lake	Tuesday Lake
Inputs			
Production	293	46	48
Nonplanktonic	—	374	72
Total input	293	420	120
Outputs			
Photodegradation	64	349	46
Sedimentation			
chlorophyll *a*	10	7	6
pheophytin *a*	57	27	31
pheophorbide *a*	34	20	37
Total	101	54	74
Decay in deep traps			
chlorophyll *a*	4	3	0
pheophytin *a*	9	11	2
pheophorbide *a*	3	3	1
Total	16	17	3
Change in standing crop	7	0	−3
Total output	188	420	120

Humic acid concentrations are greatest in Tuesday Lake and least in Peter Lake (Elser 1987).

Photo-oxidation rates of *a*-phorbins were broadly similar to those calculated in 1984 (Carpenter et al. 1986) and in other studies (Welschmeyer and Lorenzen 1985a). Interannual and interlake differences in rates of bleaching may have resulted from changes in algal community composition, but were unrelated to humic acid levels. Red light (>600 nm) can photodegrade chlorophyllous pigments, but is not absorbed by humic compounds. Although pheophorbide *a* kinetics were slower than those of other *a*-phorbins, photodegradation rates were still sufficient to completely bleach pheophorbide *a* standing stocks within a week, except in Tuesday Lake (Table 3).

Alloxanthin losses due to hypolimnetic decay were intermediate to those of other carotenoids (Fig. 6) and were not capable of producing the observed excess in sediment traps. Similarly, alloxanthin could not be produced through degradation of other carotenoids because the relative rates of carotenoid decay showed no consistent pattern among lakes, yet alloxanthin was overrepresented in the traps of all lakes. In contrast, pheophorbide *a* corrections were usually lower than those of other *a*-phorbins, suggesting that overrepresentation of pheophorbide *a* in traps was partially the result of preferential preservation. Interlake variation in hypolimnetic decay reflects differences in bacterial and microfaunal activity (Leavitt and Carpenter 1990).

TABLE 2. Epilimnetic budget of chl *a* in Paul, Peter, and Tuesday Lakes in 1986 ($\mu mol \cdot m^{-2}$). Calculated from equation (2). Epilimnion defined by depth of 25% surface irradiance.

	Paul Lake	Peter Lake	Tuesday Lake
Inputs			
Production	213	13	15
Nonplanktonic	—	272	246
Total input	213	285	261
Outputs			
Photodegradation	44	199	28
Sedimentation			
chlorophyll a	9	11	45
pheophytin *a*	43	34	98
pheophorbide *a*	25	20	83
Total	77	65	226
Decay in shallow traps			
chlorophyll a	3	5	0
pheophytin *a*	7	13	7
pheophorbide *a*	2	3	1
Total	12	21	8
Change in standing crop	1	0	−1
Total output	134	285	261

Three lines of evidence indicate that the overrepresentation of alloxanthin in sediment traps was caused by rapid sedimentation of cryptophytes in zooplankton feces. First, estimates of pigment ingestion by herbivores showed that 25–75% of alloxanthin standing stock was consumed per week (Fig. 7). Ingestion of alloxanthin was at least four-fold greater than that of other pigments using the exclusive grazing model (Fig. 7a). Using the inclusive model, alloxanthin ingestion exceeded that of all other pigments, except fucoxanthin in Peter Lake (Fig. 7b). Algal carotenoids survive gut passage (Repeta and Gagosian 1981, 1982; Barlow et al. 1988) and are less degraded than nonpigmented organic matter (Leavitt and Brown 1988). In fact, alloxanthin may be better preserved than chlorophyllous pigments (Klein et al. 1986). Therefore, high ingestion combined with low degradation can increase sedimentation of alloxanthin relative to other carotenoids.

Second, photo-oxidation of alloxanthin should have completely degraded standing stocks before cryptophytes could settle out of the photic zone, even under the most rapid sinking rates possible (Table 3). This means that rapid production of alloxanthin relative to other carotenoids cannot produce the excess of alloxanthin in the traps. Instead, the observed high sedimentation of alloxanthin can be explained only if sinking rates exceed those of individual moribund cells. Incorporation of alloxanthin into rapidly-sinking feces of large grazers could produce the rapid sinking rates required. The same argument has been used to explain increased sedimentation and decreased photodegradation of chlorophylls in the presence of large grazers (Welschmeyer and Lorenzen 1985a, b; Carpenter et al. 1986, 1988).

TABLE 3. Half-lives ($T_{1/2}$, days) of pigment photo-oxidation and residence time in the photic zone, assuming either maximum sinking rates (spherical cell, $\phi = 1$, no turbulence) or minimum sinking rates ($\phi = 4$, continuously mixed) (Reynolds 1984). L = Paul, R = Peter, T = Tuesday Lake. Pheo *a* = pheophytin *a*, Pheob *a* = pheophorbide *a*.

	Photo-oxidation (d)[a]			Sinking time (d)[b] $\phi = 1$, Static			$\phi = 4$, Turbulent		
	L	R	T	L	R	T	L	R	T
Alloxanthin	0.47	0.18	1.45	5.00	7.58	2.71	33.03	42.05	15.03
Fucoxanthin	0.64	0.43	—	0.56	0.72	0.26	3.11	3.97	1.41
Lutein–Zeaxanthin	0.60	0.61	0.92	1.55	1.97	0.70	8.57	10.90	3.88
Chl *a*	0.79	0.23	0.57						
Pheo *a*	1.37	0.25	0.47						
Pheob *a*	2.02	0.57	6.65						

[a]For ca. 50% full sunlight (30 $E \cdot m^{-2}$).
[b]Assumes spherical cells with radius calculated from mean cell volume, cell density = 1075 $kg \cdot m^{-3}$ and temperature = 20°C.

Finally, sediment-trap material was enriched with both alloxanthin and pheophorbide *a* (Fig. 3). Carpenter et al. (1986, 1988) showed that increased deposition of all chlorophyllous pigments results from grazing by zooplankton and rapid sedimentation. While the relative concentration of pheophorbide *a* in our traps may be increased through in situ degradation of chl *a* and pheophytin *a*, pheophorbide *a* is most commonly produced during herbivory by zooplankton (Carpenter and Bergquist 1985). Therefore, parallel enrichment of alloxanthin and pheophorbide *a* suggests that grazing is the common mechanism regulating deposition.

Fecal vectors have less influence on lutein–zeaxanthin sedimentation in our lakes because the chlorophytes and cyanophytes are poorly ingested by zooplankton (Fig. 7). It is unclear whether fucoxanthin sedimentation is increased by fecal transport because of large differences between the exclusive and inclusive grazing models. Also, some herbivorous zooplankton convert fucoxanthin into chromatographically-separable derivatives during gut passage (Repeta and Gagosian 1981, 1982). In other lakes, alloxanthin sedimentation is maximal during periods of intense herbivory, while fucoxanthin settling peaks only after diatom blooms (Hurley 1988).

Chlorophyll Budgets

Comparison of chlorophyll budgets (Tables 1, 2) with previous studies in these lakes (Carpenter et al. 1986, 1988) also documented the importance of grazing by large herbivores as determinants of pigment flux. In 1985, whole-lake manipulation restructured the zooplankton community in Tuesday Lake and resulted in increased sedimentation of chlorophyllous pigments as *Daphnia* replaced *Bosmina* (Carpenter et al. 1988). In 1986, the abundance of *Daphnia* continued to increase (Elser and Carpenter 1988) and sedimentation of *a*-phorbins remained two-fold greater than in 1984, while pigment loss to photo-oxidation declined slightly relative to 1985 (Tables 1, 2).

In contrast, the reciprocal changes occurred in Paul Lake in 1986: mean daphnid densities declined (Elser and Carpenter 1988), photodegradation increased, and pigment sedimentation was lower than in 1985 (Tables 1, 2; Carpenter et al. 1988). Because the rate of phorbin photo-oxidation in Paul Lake was lower in 1986 than 1984, yet total photo-oxidation increased, our results corroborate the conclusions of Carpenter et al. (1988). Grazing by large cladocerans reduces photo-oxidative pigment loss both by increasing the rates of pigment sedimentation (in feces) and reducing standing crops available for bleaching. Therefore, reduced *Daphnia* densities in Paul Lake in 1986 allowed a higher proportion of chlorophyll standing stock to be bleached, despite lower rates of loss. Constancy of the budget terms among years provides further support that large herbivores can regulate carotenoid composition in sediment traps through selective grazing.

TABLE 4. Mean annual sediment–trap ratio (deep/shallow catch) of *a*-phorbins, and proportion (%) of total chlorophyll production and photodegradation occurring in epilimnetic waters in 1985 (from Carpenter et al. 1988) and 1986.

	Paul Lake		Peter Lake		Tuesday Lake	
	1985	1986	1985	1986	1985	1986
Trap ratio	2.08	1.31	3.03	0.82	0.85	0.33
Percent production	62.7	72.6	74.9	29.0	84.6	30.3
Percent photo-oxidation	74.0	69.0	72.5	57.1	66.1	60.9

Despite these qualitative similarities, significant interannual variation occurred between mass–balance budgets constructed 1984 to 1986. As in previous years, chlorophyll production was greatest in Paul Lake (Fig. 9). However, because production in Paul Lake in 1986 was greater than in either 1984 or 1985, while total output declined, nonplanktonic pigment input was negligible, unlike Peter or Tuesday Lakes (Tables 1, 2) or previous years (Carpenter et al. 1986, 1988). This result is surprising given that deep-sediment-trap catch usually exceeded that of shallow traps in Paul Lake, but never in Tuesday Lake, and only occasionally in Peter Lake (Fig. 4). Greater accumulation in deep traps relative to shallow is often a signal of resuspension and focusing of littoral sediments to deeper waters (Hilton 1985).

Interannual changes in the vertical distribution of pigment production can account for the discrepancy between mass–balance and sediment-trap estimates of nonplanktonic pigment input (Table 4). In 1985, 60–85% of chlorophyll production occurred in epilimnetic waters (25–100% surface irradiance) in all three lakes (Carpenter et al. 1988), whereas in 1986 this was only true of Paul Lake (Table 4). Because shallow traps were suspended in waters receiving 1–3% surface irradiance, the shift of production to deeper water (1–25% irradiance) in Peter and Tuesday Lakes reduced the proportions of pigment standing

stocks exposed to high light. The vertical shift in pigment distribution increased loading to shallow traps and reduced deep/shallow trap ratios, despite the fact that nonplanktonic inputs remained high. Pigments produced just above shallow traps were removed (in traps) prior to significant degradation, while pigments which were not collected degraded before settling into deep traps. This trend was amplified because the proportion of photo-oxidation occurring in deep waters increased in all lakes relative to 1985 (Table 4).

In contrast, the majority of chlorophyll production in Paul Lake remained well above shallow traps, where photo-oxidation could completely bleach pigments prior to sedimentation (Table 3). Under these conditions, pigment deposition is controlled primarily by zooplankton grazing and sedimentation of fecal material. Migrating zooplankton spend daylight hours below shallow traps (Dini and Carpenter 1988), therefore their contribution to deep traps is greater than that to shallow traps. As a result, mean trap ratios remained above unity (Table 4). This interpretation is supported by the observation that alloxanthin trap ratios are the highest of all pigments in Paul Lake (Fig. 4), yet alloxanthin is the most susceptible to photodegradation (Fig. 5, Table 3).

Sediment focusing should selectively increase deposition in deep traps relative to shallow (Hilton 1985). Therefore, the fact that nonplanktonic inputs remained negligible in Paul Lake, regardless of whether photic zone or epilimnetic budgets were calculated (Tables 1, 2), suggests that mass–balance equations are more accurate estimators of nonplanktonic pigment inputs than are trap ratios. This conjecture is supported by the observation that alloxanthin trap ratios indicated strong sediment focusing (Fig. 5), yet alloxanthin has no terrestrial contribution and is in low concentration in littoral sediments (Leavitt and Carpenter 1989). Based on these results, we conclude that the factors that affect pigment sedimentation are different from those that regulate flux of bulk organic matter: trap ratios of pigments are influenced not only by nonplanktonic inputs, but also by the vertical zonation of phytoplankton communities and migration of herbivores.

Implications for Paleolimnology

Our results have important implications for paleolimnological studies. Carotenoids measure different aspects of algal populations than do biovolume estimates of phytoplankton abundance. Variation in both pigment–biovolume correlations and estimates of the relative abundance of algal groups resulted from taxon-specific differences in cellular pigment content, species replacements, lack of variation in algal abundance, and the influence of nonplanktonic inputs. Thus, fossil carotenoid profiles are influenced by both algal community composition and the factors that regulate pigment sedimentation.

Details of fine-scale (weekly–monthly) changes in epilimnetic carotenoid standing crops are lost during sedimentation. Consequently, fossil carotenoids may not monitor short-term changes in algal abundance. This suggests that the minimum temporal resolution of fossil carotenoid profiles may be one year, a result consistent with fine-interval analyses of sediment cores (Leavitt and Carpenter 1989 and unpubl. data).

Sedimentation of carotenoids in these unproductive lakes is strongly influenced by complex interactions between photo-oxidation and herbivore grazing, as is the deposition of chlorophyllous pigments (Carpenter et al. 1986, 1988). For all pigments, photo-oxidative losses are sufficient to substantially degrade standing stocks by the time moribund algae sink out of the photic zone. However, carotenoids in small, easily-ingested cells can enter the rapidly-sinking feces of large zooplankton and thereby avoid bleaching. As a result, the pigment composition in sediment traps is influenced by herbivory and is biased towards carotenoids contained in rapidly-sinking particles. This conclusion is substantiated by fossil carotenoid profiles from these lakes. Concentrations of carotenoids in sediments increased dramatically following transitions from *Bosmina*-dominated communities to those composed of large-bodied *Daphnia* (Leavitt et al. 1989). We do not know if the relative importance of degradative processes changes in more productive lakes. However, our explanation is consistent with the excess of otherwise labile alloxanthin in both marine (Repeta and Gagosian 1987) and other freshwater sediments (Hurley 1988).

We predict that in the absence of change in zooplankton community structure, changes in fossil alloxanthin concentration will primarily monitor altered light regimes. This hypothesis can best be tested in manipulated lakes in which the history of experimentation has been documented and changes in algal community composition are known.

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The Lobster (*Homarus americanus*) Fishery off Lower Argyle, Southwestern Nova Scotia

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Campbell, A. 1990. The lobster (*Homarus americanus*) fishery off Lower Argyle, southwestern Nova Scotia. Can. J. Fish. Aquat. Sci. 47: 1177–1184.

Catch and effort data for the lobster (*Homarus americanus*) fishery of Lower Argyle, Nova Scotia, were obtained from sales slips on a weekly basis during 1978–87. Lobster size–frequency distributions were collected during at-sea sampling at the beginning and end of each fishing season. This fishery experienced a three-fold increase in landings (from 87.6 to 270 t) due to an increase in recruitment during the 10-yr study. The fishermen responded to the improved recruitment by increasing the total number of days fished per season by 41%. Overall mortality of recruited lobster (estimated from size–frequencies) rose from 55% in 1978 to 72–81% during 1985–87. During the 1987–88 fishing season, about 1 t lobster were removed per km^2 of fishing grounds. Regression relationships between a prerecruit juvenile abundance index (numbers of lobster/trap haul) and the recruited yield one to two fishing seasons later were significantly correlated for 8–9 yr of data; equations predicted that lobster yields would remain high during the 1988–89 fishing season but decline in this area during 1989–90 fishing season. Management implications of forecasting recruitment yields 1–2 yr in advance and possible strategies to reduce major recruitment fluctuations in this fishery are discussed.

Les données sur l'effort de pêche et les prises de homards dans la pêcherie de Lower Argyle, en Nouvelle-Écosse, ont été compilées chaque semaine à partir des bordereaux de vente de 1978-1987. Au début et à la fin de chaque saison de pêche, les distributions de fréquence de taille des homards ont été établies lors d'échantillonnages en mer. Les débarquements de cette pêcherie ont triplé (passant de 87,6 t à 270 t) en raison d'une augmentation du recrutement pendant les 10 années de l'étude. Les pêcheurs ont réagi à ce phénomène en augmentant le nombre total de jours de pêche par saison de 41 %. Le taux de mortalité global de ces nouvelles cohortes (estimé à partir des fréquences de taille) est passé de 55 % en 1978 à 72 à 81 % de 1985-1987. Pendant la saison de pêche de 1987–1988, près d'une tonne de homards a été pêchée par km^2 de pêcherie. Les relations de régression entre un indice d'abondance des cohortes juvéniles pré-recrutement (nombre de homards/casier halé) et le rendement en recrues une ou deux saisons de pêche plus tard étaient corrélées significativement pour 8–9 années de données; les équations ont prédit que les rendements en homards resteraient élevés pendant la saison de pêche 1988–1989, mais diminueraient dans ce secteur au cours de la saison suivante. Les incidences des prévisions des rendements du recrutement 1 ou 2 ans à l'avance sur la gestion et les stratégies pouvant être adoptées pour réduire les fluctuations importantes du recrutement dans cette pêcherie sont examinées.

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Coastal waters off southwestern Nova Scotia have traditionally supported an important lobster (*Homarus americanus*) fishery (Campbell 1989a). To assist the stock assessment of lobster in the southern half of this area, the Lower Argyle lobster fishing grounds (Fig. 1) were chosen for study because the locality was accessible and considered representative of the local fishery. For 10 yr (1978–88), fishery characteristics, such as catch-per-unit-effort (CPUE) and size–frequency information of lobster caught by traps at sea, were monitored during each fishing season. No equivalent long-term fishery information has been available from the Lobster Bay area (Paloheimo 1963; DeWolf 1974; Stasko and Campbell 1980; Campbell 1982, 1983a, b; Campbell and Pezzack 1986), one of the most productive lobster fisheries in Canada. During 1980–88, a major recruitment pulse occurred in southwestern Nova Scotia (Lobster Fishing Area, LFA#34), resulting in an increase in lobster landings not observed since the turn of this century (Fig. 2). Lower Argyle landings represent about 3–5% of the total annual lobster landings from LFA#34. In this paper I describe the characteristics of the Lower Argyle lobster fishery to show how catch and effort trends, size frequencies, and total mortality rates of recruited (commercial-sized) lobsters have changed during the 1978–87 fishing seasons, and attempt to develop an equation using trap-caught prerecruits to predict recruited yield 1–2 yr in advance.

Materials and Methods

The Lower Argyle lobster fishing area (Fig. 1) is approximately 280 km^2 with depths ranging from 3.6 to 36.6 m. Fishing effort such as the number of licences and maximum traps per licence (375), has been limited since the late 1960's. The fishing season (last Tuesday in November to 31 May) is intended to protect lobster while molting, mating, and extruding eggs during the summer and to supply fresh lobster when they are

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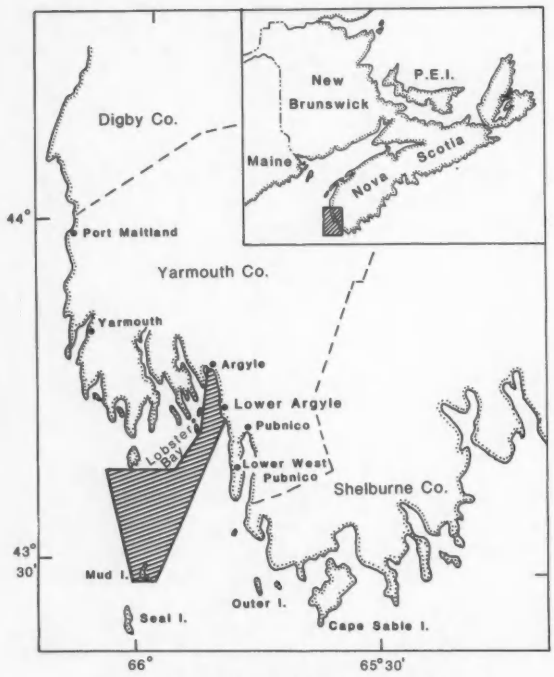

FIG. 1. Lower Argyle fishing area (shaded).

in scarce supply from other areas. Egg-bearing females are protected, and a legal minimum size allows only lobsters 81 mm (3 3/16 inches) carapace length (CL) or longer to be removed by the fishery. Inshore lobster fishermen are allowed to set traps within 32 km from shore, beyond which grounds are reserved for an offshore lobster fishery that has developed since 1971 (Pezzack and Duggan 1983).

Many commercial lobster traps were wooden, either box-like or half-cylindrical in shape, 80–122 cm long, with the base wider than the top, with three to four bows, with a variety of entrance rings 11–26 cm in diameter, (some trap entrances are made only of knotted twine instead of wood or plastic entrance rings) and lath spaces 2.6–4.0 cm wide (data from Lower Argyle fishermen's traps measured May 1979–May 1981). Recently, traps with plastic-coated wire are being used more commonly than before by fishermen.

Commercial lobster landings, by weight, were recorded on sales slips and collected during 1978–87. During 1978–85, catch-per-unit-effort (CPUE) data were also obtained from the Lower Argyle lobster cooperative record books. All fishermen from Lower Argyle belonged to the cooperative.

At the beginning (November–December) and end (May) of each fishing season, observers went to sea on commercial boats and recorded the CL and sex of all lobster caught in traps. The number of traps hauled, trap depth, and soak time (number of days a trap was in the water between hauls), and the bottom and surface water temperatures were also recorded. Soak time ranged between 1–3 d and was not considered to affect the catch or retention rates of the traps. The weights (g) of a wide size range (50–160 mm CL) of male and female lobster were measured during the 1980–81 fishing season.

As size frequency information for December 1982 was not available from the Lower Argyle area, a sample from the adjacent area fished by Lower West Pubnico fishermen was substituted.

Data Analyses

Catch was defined as the yield (kg) of lobster landed per week or per fishing season. Fishing effort was measured by two methods: (1) the number of traps hauled per day from the at-sea samples; and (2) the number of days fished per week or fishing season by a fisherman. The latter was obtained from the number of times lobster sales were recorded on the sales slips; the majority of fishermen sold their catch on the same day as traps were fished, as determined from interviews with fishermen. However, during the 1987–88 fishing season, many fishermen stored their lobster for over a week before selling them; therefore, days fished per fishermen could not be estimated from the sales slips for this period.

Linear regression analyses (Nie et al. 1975) were used to examine the relationship between (1) total days fished and yield per fishing season, and (2) $\log_e$ weight (W in g) and $\log_e$ carapace length (L in mm) for male and female lobster. From the latter, a power regression for males was estimated as $\log_e W = -7.2474 + 3.024 \log_e L$ ($N = 180$, $R^2 = 0.98$), and for females as $\log_e W = -7.0482 + 2.985 \log_e L$ ($N = 190$, $R^2 = 0.99$). The weight of each lobster in the legal catch curve data was calculated from the weight–length regressions; consequently size frequencies for fall and spring of each fishing season, according to total lobster weights in the sample, were constructed. An overall weighted size frequency for a whole fishing season was obtained by multiplying the proportion of total landings for a particular 2-wk period with the appropriate size frequency (by weight) and subsequently summing the weighted size frequencies.

Each fishing season is referred to by the year in which the season began, e.g. the 1981 season was from November 1981 to May 1982.

Overall mortality rates (sexes combined) for the first molt group (m) were estimated for each fishing season from the weighted size frequencies (Caddy 1977; Campbell 1980).

$$(1)\quad Z_m = (\log_e N_m - \log_e N_{m+1})/\Delta t_m,$$

where, N_m = number in molt group, Δt_m = mean intermolt duration at the mean size of molt group m, Z_m = instantaneous total mortality coefficient of molt group m. Molt group sizes (sexes combined) were m = 81–94 mm CL and $m+1$ = 95–109 mm CL (after Campbell 1983a). The intermolt duration (Δt_m) for the first molt group was assumed to be 1.0 yr in the absence of data for the area. The overall mortality (A_m) for each molt group (percent) was calculated as:

$$(2)\quad A_m = (1 - e^{-Zm}) \cdot 100.$$

Natural mortality (M) for recruited lobsters is generally observed to be about 0.1 or 10% (Campbell 1980). Overall mortality includes fishing mortality (F) and natural mortality, $Z = F + M$.

In an attempt to develop a predictive relation between pre-recruits and recruits, the following equation was developed to estimate the constants a and b with the linear least squares method:

$$(3)\quad \log_e R_m = \log_e a + b \log_e P_{m-x},$$

where, R_m = recruited yield (t) which was estimated by multiplying the proportion in molt group m (81–94 mm CL of

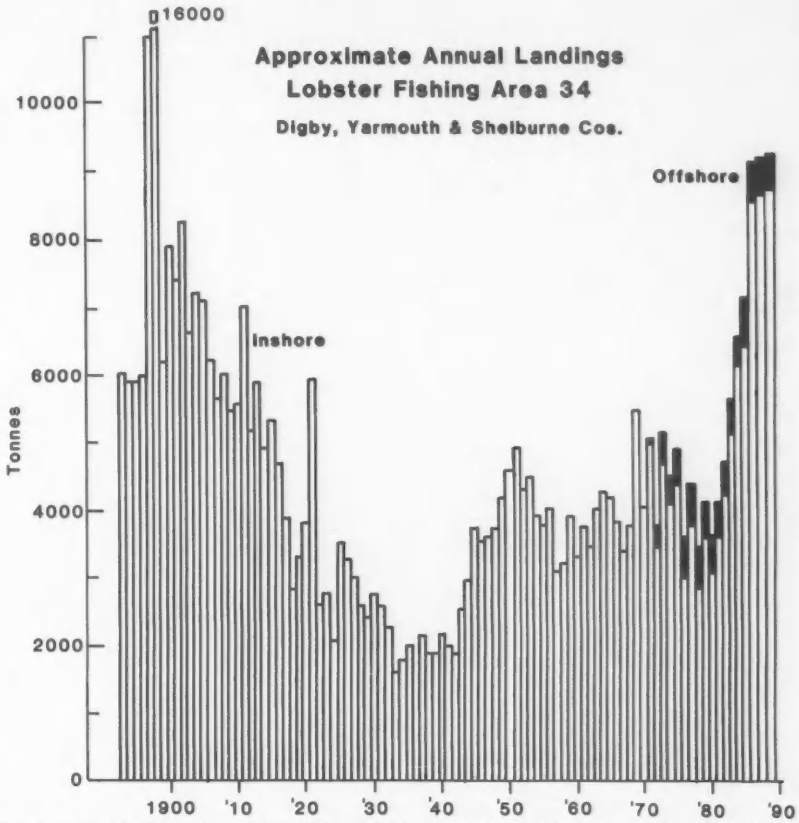

FIG. 2. Annual landings from lobster fishing district 34 (includes Digby, Yarmouth and Shelburne counties); shaded columns represent the offshore fishery.

the weighted size frequencies in Table 2) by the total landed lobster weight (t) during a fishing season, and of abundance, P_{m-x} = the "prerecruit juvenile index," or the number of prerecruits per trap haul in molt group, $m-x$ (Table 1). When $x=1$, the prerecruits (68–80 mm CL) from the last 2 wk of May (e.g. 1980) were related to the recruited (81–94 mm CL) biomass of the next fishing season (e.g. 1980). When $x=2$, the prerecruits (56–67 mm CL) from the last 2 wk of May (e.g. 1980) were related to the recruited (81–94 mm CL) biomass of the following second fishing season (e.g. 1981). Molt groups were estimated from Campbell (1983a) and all lobsters in the 56–94 mm CL range were assumed to molt about once per year during July–October (i.e. in between fishing seasons).

Results

Catch and Effort

Landings for each fishing season increased from 80.8 to 290.4 t during 1978–87 (Fig. 3A). Although the number of fishermen (= number of boats) rose slightly from 37 to 42 by 1985, the number decreased to 35 by 1987. In contrast, the total number of days fished per season rose from 1416 in 1979 to about 2072 in 1982 and remained constant at approximately 2000 thereafter (Fig. 3A). The total yield per fisherman (= boat) for the whole season and per day increased each season as did the number of days fished per fisherman (Fig. 3B). The total seasonal yield ($y=t$) was correlated with the total number of days fished (X) according to $y=254.8X-282500.3$ ($R^2=0.82$, $P<0.01$, $N=9$); however the increase in days fished probably resulted from higher yields rather than the reverse.

Although the highest landings per week were recorded during the first few weeks of each season (Fig. 4A), only $44.9\% \pm 7.8$ SD of the total landings were made during the November–February period compared with $55.1\% \pm 7.8$ SD ($N=10$) during March–May. Although all fishermen were active during fall (November–December) and spring (March–May) more effort (days fished) was required to catch losters in the spring compared with the fall; a mean $76.4\% \pm 4.6$ SD of the total days fished were made during spring compared with $23.6\% \pm 4.6$ SD ($N=9$) during fall (see also Fig. 4B). The low winter temperatures and storms reduced lobster catchability (McLeese and Wilder 1958) and fishing activity during winter (January–February). The CPUE generally declined during fall, then increased slightly in the spring (Fig. 5).

Size-Sex Frequencies

Lobsters at sizes of 60–95 mm CL were abundant in traps off Lower Argyle where the minimum legal (recruit) size is 81 mm CL (Fig. 6A). There was an increase in the number of prerecruits (shorts) per trap haul and general increase in the

TABLE 1. Summary of lobster size frequencies from at-sea samples taken in the Lower Argyle fishing area during 1978–87. * = From Lower West Pubnico area since no Lower Argyle information available.

			Prerecruits			Recruits						
				Number per trap haul					Number of females			
Date	Number of trap hauls	Total number of lobster	Percent of total	56–57 mm CL P_{m-2}	68–80 mm CL P_{m-1}	Number per trap haul	Mean CL (mm)	Mean weight (kg)	Percent of total recruits	Berried per 1000 trap haul	Water temp. (°C)	Mean depth (m)
7 Dec. 1978	129	154	48.0	0.08	0.49	0.62	90.8	0.62	51.2	0	5.8	24
17–30 May 1979	713	628	54.1	0.08	0.40	0.38	94.8	0.72	41.3	19.6	8.0	24
29 Nov.–3 Dec. 1979	404	1111	53.3	0.25	1.21	1.28	89.8	0.59	40.3	2.5	7.5	20
16–28 May 1980	989	1226	73.2	0.15	0.76	0.31	96.2	0.76	45.0	23.3	8.1	18
25 Nov. 1980	340	1143	47.5	0.19	1.41	1.76	90.1	0.60	37.5	2.9	7.1	8
20 May 1981	374	781	76.8	0.26	0.75	0.49	92.9	0.68	35.9	5.3	9.1	22
2–8 Dec. 1981	425	1386	53.0	0.08	1.64	1.53	88.3	0.56	40.0	7.1	6.8	20
18 May 1982	335	603	71.5	0.22	1.07	0.50	91.5	0.64	35.5	17.9	6.0	20
8 Dec. 1982*	316*	939*	55.7*	0.16*	1.49*	1.32*	88.9*	0.57*	45.7*	0*	7.5*	0*
26–27 May 1983	246	484	67.8	0.17	1.16	0.61	92.8	0.67	50.6	24.4		
1–6 Dec. 1983	683	1707	50.8	0.07	1.20	1.23	88.7	0.57	35.0	1.5	5.5*	18*
16 May 1984	376	679	74.8	0.27	1.08	0.45	89.6	0.59	46.8	5.3	6.5*	22*
12–13 Dec. 1984	543	1309	65.8	0.22	1.37	0.82	88.5	0.57	43.8	1.8		
24 May 1985	181	472	89.8	0.31	2.02	0.27	89.0	0.58	52.1	0	9.0	16
6 Dec. 1985	317	967	75.3	0.33	1.96	0.75	86.4	0.52	36.8	6.3	5.0	20
14 May 1986	361	799	83.8	0.24	1.62	0.35	89.6	0.59	49.6	16.6	7.0	20
9 Dec. 1986	311	1471	77.2	0.41	3.23	1.07	87.2	0.54	39.1	6.4		
21 May 1987	386	1087	78.5	0.27	1.94	0.57	90.1	0.60	52.6	36.3	7.1	22
4 Dec. 1987	379	1930	66.8	0.27	3.13	1.69	87.3	0.54	29.5	0	5.8	10
20 May 1988	395	1112	69.0	0.10	1.85	0.84	92.0	0.65	42.0	35.4	6.9	8

proportion of prerecruits compared with the total lobsters caught during 1978 to 1987 (Table 1). The size frequencies of recruited lobsters, weighted according to fall and spring catches (e.g. Fig. 6B), indicated that there was a shift to a smaller median carapace length during 1978–87 (Table 2).

Removal of lobsters due to commercial fishing caused the number of recruits caught per trap haul to be lower in the spring than during the fall of each fishing season (Table 1). The mean CL and weight of lobsters were invariably larger in the spring compared with the fall (Table 1) which was due to a proportional increase in the relative number of large (>95 mm CL) lobsters caught in traps during the spring compared with the fall season. This also was reflected in the larger number of berried females caught in every spring (except May 1985) compared with the fall of each fishing season (Table 1). The sex ratio was approximately 1:1 for the prerecruits for every fall and spring period. However, the percentage females of total recruits in the fall was usually lower (except for 1978, 1980, and 1981) than during the spring season (Table 1). The recruit data indicate that more males than females were captured in most falls and that although there was a shift to proportionally more females being captured in the spring than fall, the sex ratio was still in favour of males during six springs while in the other few springs the sex ratios were close to 1:1 (Table 1).

Mortality Estimates

The overall mortality estimates based on the weighted size-frequencies showed an increasing trend of 55.4 to 80.6% during 1978–85, and decline to 72.3% during 1987 (Table 2).

Prerecruit Abundance–Recruitment

A significant correlation ($R^2 = 0.834$, $P < 0.01$, $N = 9$) was obtained between the prerecruit abundance index (first molt prior to recruitment) and the recruited yield for the following fishing season $\log_e R_m = 4.828 + 0.890 \log_e P_{m-1}$ (standard error of estimate = 0.22) (Fig. 7A). A similar correlation ($R^2 = 0.763$, $p < 0.01$, $N = 8$) was obtained between the prerecruit index (two molts prior to recruitment) and the recruited yield two fishing seasons later $\log_e R_m = 6.352 + 0.835 \log_e P_{m-2}$ (SE = 0.22) (Fig. 7B). Both regressions predicted that the recruitment yield would remain high for the 1988–89 fishing season (215.6 and 191.6 t, respectively, 95% confidence limits are 166.2–279.6 and 150.9–243.3) similar to the previous few years, based on the high prerecruit abundances recorded for P_{m-2} during May 1987 and P_{m-1} during May 1988 (Table 1). However, recruitment yields for the 1989–90 fishing season were predicted to be less than half (81.2 t, 95% confidence limits are 56.3–117.2) of the last few years, assuming that P_{m-2} value (0.10) from May 1988 (Table 1) is accurate. Additional sea samples from Lower West Pubnico (Abbotts Harbour) during 18–21 May 1988 indicated similar catch rates of 0.11 for P_{m-2} and 1.39 for P_{m-1} (number of trap hauls = 726).

Discussion

The Lower Argyle lobster fishery experienced an increase in recruitment and corresponding increase in lobster landings during the 10-yr study (1978–87). The recruitment increase was evidenced by (1) increases in the number of prerecruits per trap haul, (2) decline in mean CL of recruits, and (3) a 308% increase

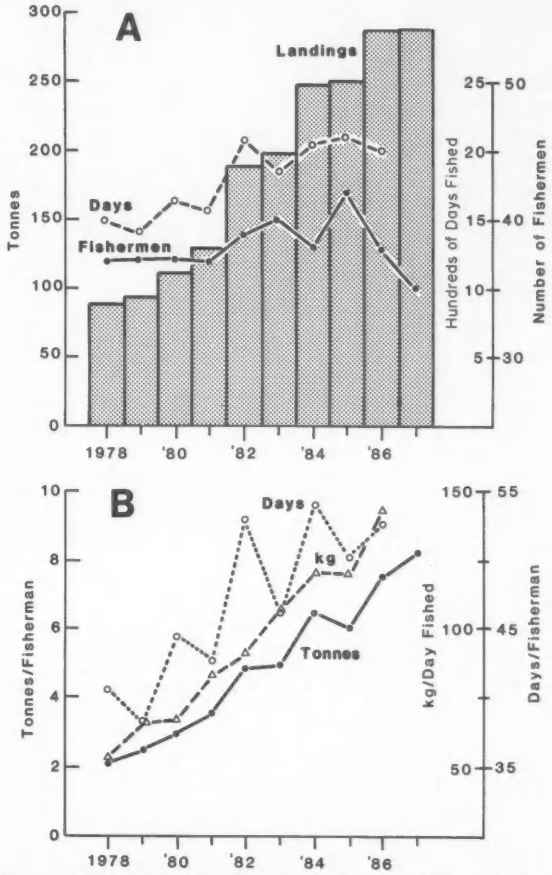

FIG. 3 (A). Trends in total landings, fishermen (=boats) and days fished, and (B) total catch, fishing days and daily catch per fisherman for each fishing season at Lower Argyle, 1978–87.

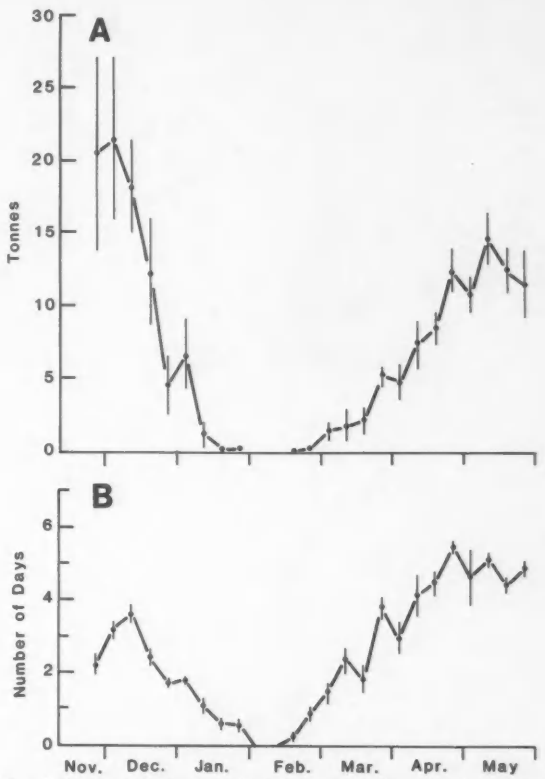

FIG. 4. Average (A) landings ($N=10$) and (B) days fished per fishermen ($N=9$) grouped by week at the Lower Argyle lobster fishery. Dots are means and vertical lines are ±1 SE.

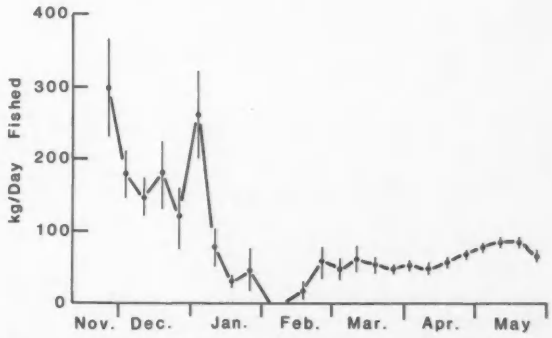

FIG. 5. Average catch per day fished (grouped by week) at the Lower Argyle lobster fishery. Dots are means and vertical lines are ±1 SE. $N=9$.

in yield when comparing average landings (88–270 t, respectively) of fishing seasons 1978 and 1979 with those of 1985 and 1986. The fishermen responded to the increase in recruitment by increasing their number of days fished by a mean of 41% (1461–2058 d): concomitantly overall mortality rates increased by 45% (55.4–80.6%) (comparing 1978 and 1985 fishing seasons).

The size frequencies must be considered in relative terms because trap data can give a biased estimate of lobster densities. Traps are difficult to standardize because catching efficiencies vary from trap to trap, area to area, and from time to time due to lobster activity levels changing with temperature (McLeese and Wilder 1958), and changes in physiological condition of lobsters (Elner 1980). Selectivity of traps, especially for prerecruits, could be affected by lath spacing (Krouse and Thomas 1975). Consequently the methods used only approximate estimates of mortality rates of recruited lobster. Although effective fishing effort is best estimated from the total number of traps hauled, unfortunately, such detailed information was not available. Clearly, caution in interpreting these results is needed, especially since the effects of variations in latent effort (Pringle and Duggan 1984), fishing power, and effective effort between years still require evaluation. Also, the use of commercial traps for estimating juvenile prerecruit abundance indices still needs to be verified and standardized in terms of accuracy, since the dimensions (e.g. escape vents) and the material used (e.g. from wood to plastic-coated wire) in these traps are being changed.

The information from the size–sex frequencies, however, provided some tantalizing evidence for the seasonal movement of lobsters especially mature females in Lobster Bay: i.e. higher numbers of males than females, lower mean CL of recruited lobsters and lower number of berried females in the fall com-

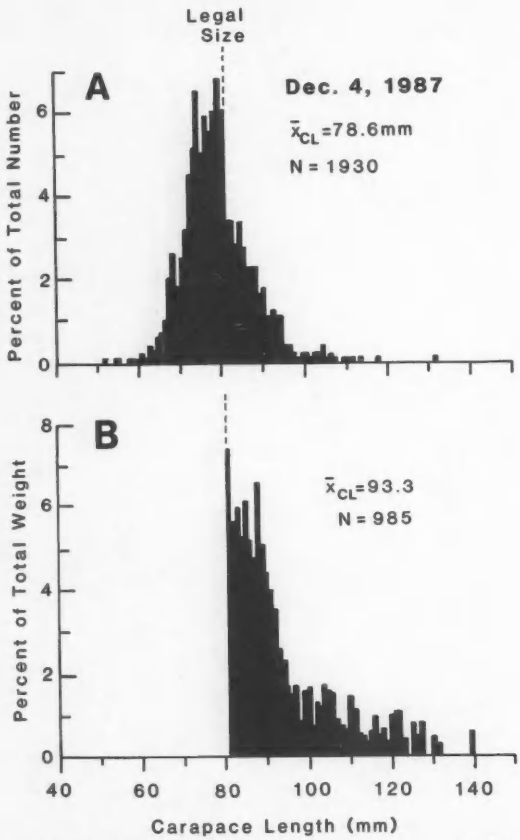

FIG. 6. Examples of size frequencies of (A) the total number of lobsters caught in 379 traps hauled on 4 December 1987, and (B) weighted size frequency according to recruited lobsters caught in samples during the 1987 fishing season in the Lower Argyle lobster fishery.

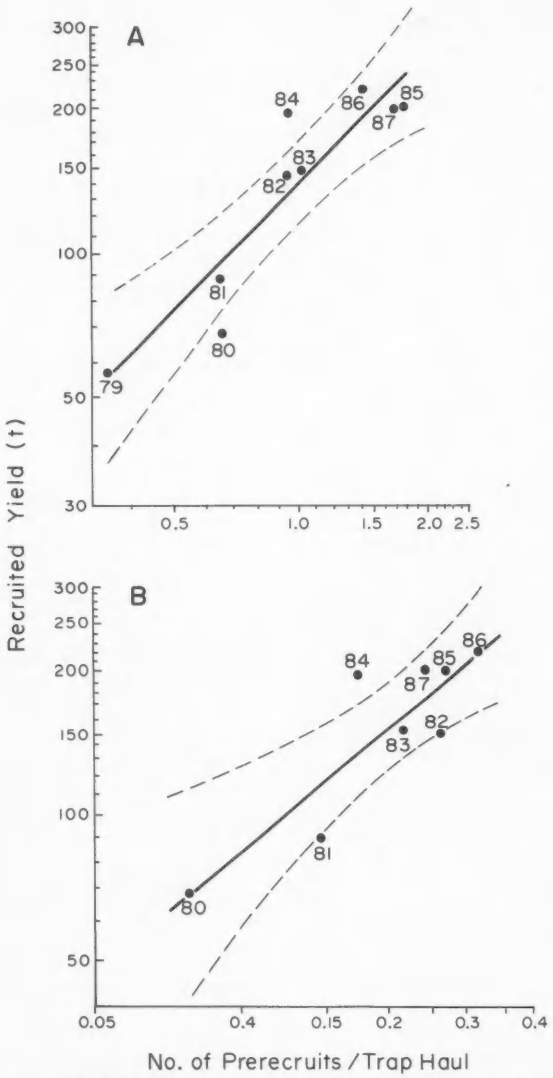

FIG. 7. Relationship between prerecruit juvenile index of abundance (A) first molt prior to recruitment, 68–80 mm CL during the last 2 wk of the previous fishing season (May) and (B) two molts prior to recruitment 56–67 mm CL during last 2 wk of May of the second previous fishing season and recruited yield (t, 81–94 mm CL) in the following fishing season. Numbers beside dots are for the fishing season year (i.e. 79 = 1979–80) of recruited yield. Dashed lines are 95% confidence limits.

pared with the spring fishery. Fishermen believe that there is an inshore movement of large "school" lobsters in southern Nova Scotia in spring (Aiken 1971). To date tagging experiments have provided little information on seasonal movements of lobsters in Lobster Bay (Campbell and Stasko 1985; Campbell 1989b) although the phenomenon was recorded in inshore areas of the Bay of Fundy (Campbell and Stasko 1986; Campbell 1986a). Also mature females moved earlier than males in the fall causing unequal sex ratios heavily in favour of males trapped off Alma (Campbell 1984). Other factors also may have caused a shift in the seasonal size–sex structure of lobsters caught in traps. The growth of large males can be faster than females after sexual maturity (Campbell 1983a) although molting of lobsters normally occurs during July–November (Campbell 1983b). Although Sharp and Duggan (1985) showed some lobster traps were distributed in deeper water in spring compared with fall off southwestern Nova Scotia, in the present study, the traps sampled belonging to Lower Argyle fishermen were distributed in the same general fishing area and depth range in both spring and fall of each fishing season (D. E. Graham, St. Andrews Biological Station, St. Andrews, N.B. E0G 2X0, pers. comm.).

The reason for the major increase in recruitment in the last few years is unknown. However, the low fishing mortality in the late 1970's should have allowed more females to produce eggs than would have happened at higher fishing mortality rates (Campbell 1985). The large number of eggs per recruit may have helped to increase recruitment into the fishery 5–8 yr later. The larger number of ovigerous females caught in the 1980's as compared with the 1970's in Lobster Bay (Stasko and Campbell 1980; Campbell and Pezzack 1986; this study) would also support this explanation. A problem, at present, is that little is

TABLE 2. Summary of weighted size frequencies of recruited lobster for each fishing season from the Lower Argyle fishing area. Fishing season e.g. 1978 = Nov. 1978–May 1979. * = Lower West Pubnico Dec. 1982 sea sample included.

	Carapace length (mm)		Percent of total		Mortality estimate	
Fishing season	Mean	Median	First molt	Second molt	Z_m	A_m
1978	94.0	91	62.6	27.9	0.8075	55.40
1979	95.8	91	60.8	27.2	0.8050	55.29
1980	95.9	91	62.3	24.9	0.9144	59.93
1981	93.4	91	67.4	23.5	1.0525	65.09
1982*	90.4	89	77.1	19.5	1.3733	74.67
1983	91.2	89	74.7	20.0	1.3183	73.24
1984	90.5	89	78.8	19.4	1.4048	75.46
1985	90.3	88	79.2	15.4	1.6378	80.56
1986	91.0	89	76.1	18.7	1.4010	75.36
1987	93.3	89	68.8	19.1	1.2838	72.30

known about the source of larvae that settle in the area, although some studies (Stasko 1978; Stasko and Gordon 1983; Harding et al. 1983; Campbell and Pezzack 1986; Harding and Trites 1988) suggest that larvae may come form berried females hatching their eggs from inshore areas of southwestern Nova Scotia, or offshore areas such as Browns Bank and Georges Bank.

The use of a prerecruit abundance index from commercial traps to predict recruitment holds some promise in forecasting lobster yields for the Lower Argyle lobster fishery. Caputi and Brown (1986) used a juvenile abundance index to successfully predict *Panulirus cygnus* recruitment in Western Australia. In the present study, although abundance indices were based on only nine or eight year-classes, the correlations were significant. The ability to forecast recruitment one to two fishing seasons ahead could allow improved financial planning by fishermen and improved responses to recruitment fluctuations by fisheries managers choosing appropriate management options. For example, should low recruitment occur, as suggested for the 1980–90 fishing season by the low P_{m-2} value for May 1988, management measures (e.g. reducing effort such as trap numbers, licences and/or fishing seasons) could be implemented at the beginning of the season to protect the potential brood stock. Industry resistance, however, could prevent regulating significant reductions in fishing effort in the short term.

The increased exploitation of lobster as recruitment increases clearly requires changes in conservation regulations to ensure that fluctuations in yield will not be amplified. A number of long-term management options have been considered using yield and egg-per-recruit analyses (Campbell 1985, 1986b). Increasing the recruit size by about one molt increment was considered the most effective regulation option for increasing yield and eggs per recruit, especially in an area where the current recruit size is one molt below onset of maturity (Campbell and Robinson 1983) and fishing effort is socio-economically difficult to reduce.

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Ovulatory Rhythm and a Method to Determine the Stage of Spawning in Atlantic Cod (*Gadus morhua*)

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The size frequency distribution of the vitellogenic oocytes in Atlantic cod (*Gadus morhua*) changes in a predictable manner as spawning progresses. This change was studied by sampling successive batches of eggs from reared cod kept in the laboratory. The shape of the vitellogenic oocyte mode was described by measuring the diameter, standard deviation, and skewness and kurtosis. Linear regressions were derived which related the portion of the total number of eggs spawned per season (PES) to the number of vitellogenic oocytes per gram of the ovary and in relation to the standard deviation of the distribution. This method which allows the PES in a spawning fish to be estimated has the following applications. For fish stock assessment purposes fecundity estimates can be made on spawning rather than prespawning fish and residence times on spawning grounds can be calculated. In addition successive batches of eggs can be identified and studied. This is important in hormonal and larval studies. A detailed description is given of the oocyte growth during the ovulatory rhythm. The rhythm consists of four periods: arrest, final growth, hydration, and ovulation. It is suggested that the egg size is determined during the final growth period.

La distribution de fréquence du diamètre des ovocytes pourvus de vitellus chez la morue (*Gadus morhua* L.) varie de façon prévisible au cours de la fraie. L'échantillonnage de groupes successfis d'oeufs chez des morues d'élevage gardées en laboratoire a permis d'étudier cette variation. Le diamètre des ovocytes pourvus de vitellus, l'écart-type, l'asymétrie et l'aplatissement ont servis à l'établissement du mode. On a aussi calculé des régressions linéaires établissant une relation entre le pourcentage du nombre total d'oeufs pondus par saison (PES) et le nombre d'ovocytes pourvus de vitellus/g présent dans l'ovaire ainsi qu'entre l'écart-type de la distribution. Cette méthode qui permet de déterminer la PES d'un reproducteur a les applications suivantes. Dans le cas d'évaluations de stock, des estimations de la fécondité peuvent être effectuées à partir de données sur les reproducteurs au lieu des pré-reproducteurs et les périodes de séjour dans les frayères peuvent être déterminées. De plus, des groupes successfis d'oeufs peuvent être identifiées et étudiés. Ceci est important pour les études sur les hormones et les larves. Une description détaillée de la croissance de l'ovocyte pendant le cycle ovarien est présentée. Celui-ci comprend quatre périodes soit le repos, la croissance finale, l'hydratation et l'ovulation. On croit que la taille de l'oeuf est déterminée au cours de la dernière période de croissance.

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During spawning the size frequency distribution of the oocytes in the ovary of the Atlantic cod (*Gadus morhua*) shows a clear distinction between previtellogenic (immature) and vitellogenic (maturing) oocytes (West 1970). As a result, cod is likely to be a determinate spawner (Hunter and Macewicz 1985) and there is no recruitment of oocytes to the vitellogenic mode during spawning. Preceding ovulation, hydration causes a massive increase in volume of the oocyte (Craik and Harvey 1987). Because of this and the high fecundity (Hislop 1984) oocytes are released in batches over a period of time (Hislop et al. 1978; Sakurai 1982; Kjesbu 1989). Thus, the vitellogenic oocyte mode is reduced as spawning progresses and changes shape in a predictable manner. In the laboratory, cod are sensitive to stress and often produce poor quality eggs; however, Kjesbu (1989) kept reared specimens which produced 17–19 batches of high quality eggs (100% fertilization). The time between successive batches (i.e. the spawning interval) was regular and correlated with water temperature.

In the present study these techniques have been used to quantify the reduction in the vitellogenic oocyte mode during spawning. The main objective was to make it possible to identify the portion of eggs already spawned within the current spawning season (PES) in fish of unknown history. Such a result will be most useful in fecundity and larval studies and in studies focusing on the hormonal patterns during spawning. Changes in the standard deviation, skewness, and kurtosis of the size frequency distribution have been supplemented by studies describing the number and size of vitellogenic oocytes during maturation and spawning using an automatic egg counter (Witthames and Greer Walker 1987). The controlled experiment made it possible to describe the ovulatory rhythm in great detail. The ovulatory rhythm is central in fish farming (Billard 1989).

Materials and Methods

Experimental Protocol

During the spring of 1987 a spawning experiment, using reared Norwegian Coastal cod, was conducted at the Institute of Marine Research, Norway. The present study was a part of this experiment as were the studies by Ulvund and Grahl-Nielsen (1988) and Kjesbu (1989). In the present study two of the prespawning specimens (Females E and F) and 12 of the spawning females (Females 1–8, 13, 14, 16, and 18) were used. The latter four females were "stressed" (Kjesbu 1989), but they were here included to strengthen the understanding of the ovulatory rhythm. Stressed females show irregular spawning intervals and low fertilization rates for the eggs. One female together with two males were kept in separate 20-m^3 chambers (details of the design are described in Kjesbu 1989). Spawned eggs were collected on a sieve (500 μm) to estimate the number of eggs shed in each batch. The mean egg diameter (50 eggs) was recorded. The stage of development was determined and the time of spawning estimated by back-calculation (cf. Kjesbu 1989). By removing the females (Females 1–8) at different points in the spawning period (using number of batches shed as an index; batch 1, 2, 4, 6, 8, 10, 12, and 16, respectively), ovaries which had expelled varying batches of eggs were collected. When a particular specimen had reached the desired batch number, the time of sampling was determined according to another schedule, i.e. focusing on the spawning interval, whereby ovaries from one to 60 h after shedding were collected.

Sampling was carried out after a blow on the head, occasionally oocytes started to run at this stage. These oocytes together with those easily stripped were designated ovulated oocytes. Their number was found as for the eggs, i.e. by using the formulae given in Kjesbu (1989).

On 31 March 1987, three spawning females, designated W1-3 (W = wild), were caught off Lofoten, Norway and used for comparative purposes. Fish length was recorded to the nearest centimetre (as for the specimens above). The otoliths were removed for age determination and stock identification (Rollefsen 1933, 1934).

All ovaries were evaluated macroscopically for an assessment of the number of hyaline oocytes present. Hyaline oocytes resemble ovulated oocytes but in contrast to them still are within their follicles. Two transverse sections (designated Sample 1 and Sample 2), each about 15 g (assumed to contain about 50 000 oocytes), were then taken from the middle right ovary lobe. In the case of reared cod, the ovary and the samples were weighed to the nearest gram and to the nearest 0.1 g, respectively. The wild cod ovaries were not weighed; sample weights were determined by displacement when placed in the fixative (15 mL assumed to equal 15 g). The samples were digested in Gilson's fluid (Bagenal 1978) at room temperature for 4–6 mo and occasionally shaken by hand. Subsequently, oocytes were washed through two sieves with mesh sizes of 1400 and 180 μm, respectively. The coarse net retained fragments of connective tissue while the fine net separated the desired fraction of oocytes from the previtellogenic oocytes (the onset of vitellogenesis occurs at about 180 μm). The sample obtained was washed into a beaker. More water was added and debris was removed by decanting. This cleaning procedure was repeated several times. Samples were stored in 2% formaldehyde. To investigate the effect of various storage times duplicate samples were kept. Samples 1,S (S = short) and Samples 2,S remained in the formaldehyde solution for about 50 d before introduction to the egg counter while Samples 2,L (L = long) remained in this solution for 590 d. Both samples from each fish were examined. If not otherwise stated, the data given below are based on Sample 1,S.

Shrinkage

To estimate shrinkage in the fixatives, oocytes from the two prespawning specimens and two of the spawning specimens (Females 2 and 5) were used. The diameters from between 30 and 50 of the fresh vitellogenic oocytes and 15 of the fresh hyaline oocytes were measured using a binocular microscope (magnification 50×). Short and long axes were recorded and the mean calculated. To follow the shrinkage in Gilson's fluid, the oocyte diameters were determined the following day after immersion and after 7, 30, 60, 90, and 120 d. After cleaning, the oocytes were suspended in the formaldehyde solution, and the diameters were again measured after 43 d.

Counting and Sizing Oocytes

Oocytes, suspended in water, were both counted and sized (area) automatically using the egg counter. The data were processed by a microcomputer, and the oocyte diameter distribution displayed in 20-μm intervals (see Witthames and Greer Walker 1987).

On the screen of the pulse height analyser, values of oocyte size were shown in different channels. A calibration scale was developed between oocyte diameter and channel number by measuring the axes of the oocytes and individually passing them through the egg counter. The scale was constructed by using oocytes of different sizes in the range 298–1017 μm. The coefficient of determination (r^2) was 0.957 (n = 27). Following examination of the samples, the oocyte sizes were found to be within the range 200–1170 μm (very few oocytes were observed below 200 μm) and consequently analysis was restricted to oocytes >200 μm. Vitellogenic oocytes, being denser, passed through the apparatus before the hyaline oocytes thus giving an effective separation. If the size distribution of the vitellogenic oocytes was clearly bimodal the limit between the modes was set by taking the channel number which showed the smallest number of oocytes. Those vitellogenic oocytes in the mode containing the larger sizes (i.e. those "budding off") were given the designation "final growth oocytes."

Samples from the two prespawning specimens contained from 150 000 to 200 000 oocytes while, for spawning specimens, the number varied from 25 000 to 100 000. The count rate, based on the number of counts in 300-s intervals (n = 408),

averaged 13.46 counts·s^{-1} (95% confidence interval: 12.66–14.26). The maximum count rate of 33 counts·s^{-1} occurred occasionally for short periods of time. Consequently, the counting accuracy always exceeded 98.9% and usually was 99.5%.

The number of a particular type of oocyte in the whole ovary was estimated by multiplying the number of oocytes in the sample by the raising factor (ovarian weight/sample weight). The result was reduced by 5% due to the presence of small amounts of debris in the sample (Witthames and Greer Walker 1987) and the ovarian wall (Buzeta and Waiwood 1982).

Materials for an Image Analysis System

Thirty specimens which had ovaries containing vitellogenic oocytes, were collected during cruises in February and March 1985 and 1986 between Finnmark and Lofoten, Norway. A small (<5 g) section of the ovary (same sampling site as in laboratory animals) was removed and fixed in a solution containing 2.5% formaldehyde, 2.5% glutaraldehyde, sucrose (70 g·l^{-1}) and 0.05 *N* dimethylarsinic acid sodium salt trihydrate (pH of solution ≈7.2) (Forberg 1982).

Image Analysis System

The semi-automatic image analysis system, Leitz ASM 68K, consists of: (1) light microscope, (2) television screen, (3) cursor pen for measurements of objects, and (4) microcomputer. Oocytes were spread on a slide (wet whole mount), and the areas were measured by following the perimeter of the cell. The printout included details of 30 oocytes from each specimen in addition to the form factor which is defined in the following manner:

$$F\text{-factor} = (4\pi \cdot \text{Area}) \cdot (\text{Perimeter}^{-2}).$$

The *F*-factor indicates how closely an object approximates to a circle; straight line = 0, square ≈0.785 and circle = 1. The mean *F*-factor of the 30 oocytes was taken as the *F*-factor for that specimen.

The diameter of 100 oocytes was measured both with the aid of the cursor and with the aid of the microscope (magnification 40×, eyepiece in a fixed position) to calibrate diameter estimates by the image analysis system.

Results

Preparatory Investigations

Measurement of axes

Not all oocytes are spherical; some are elliptical. In mounting, the long axis will consequently be horizontal (like a hen's egg on a table) and the image analysis system will measure the largest area of the oocyte. In the egg counter, however, the orientation of the oocyte is unknown. To investigate this anomaly, the two extreme cases with all oocytes either showing the long or the short axis were examined.

The *F*-factors varied from 0.989 to 1.0 and the mean was 0.997 (SD = 0.003). The mean value of the coefficient of variation was 0.925% (SD = 0.726). On average, 17% of the oocytes departed from the spherical form. Departure did not appear to be related to a particular size of oocyte. Therefore, assuming that the long axis mean oocyte diameter is 600 μm (a typical value), by calculation, using the mean *F*-factor and the formula for the perimeter and for the area of an ellipse, the short axis mean oocyte diameter would be 577 μm, a diameter difference of 4%.

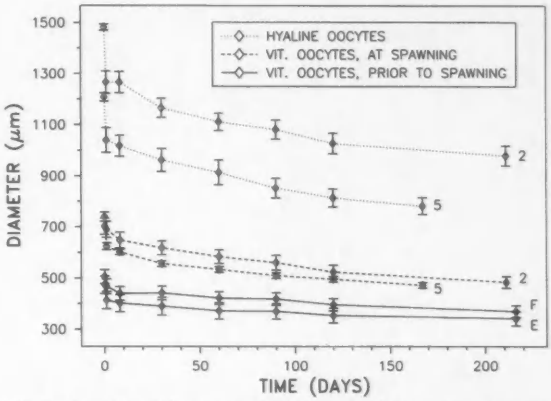

FIG. 1. Shrinkage of the oocytes in Gilson's fluid. Letter/number refers to the female from which the sample was taken. Vertical bars represent 95% confidence limits.

Measurement of oocyte diameter

The sample for which two methods were used to estimate oocyte diameter, showed a *F*-factor of 0.995 and a coefficient of variation of 1.85%. When comparing the measurements from image analysis with those from microscopy, it was noted that the image analysis system reduced the mean diameter by about 3.5% while increasing the standard deviation. A Student *t*-test with unequal variances showed, however, no significant difference ($P > 0.50$).

Measurement of oocyte shrinkage

The oocytes shrank in Gilson's fluid (Fig. 1). A total shrinkage of about 30% was observed; vitellogenic oocytes, prespawning specimens: 27.5% (mean value); vitellogenic oocytes, spawning specimens: 33.5%; hyaline oocytes: 34.5%. Apart from one of the vitellogenic samples (Female 2), one-third to one-half of the shrinkage took place within the first day in the fixative. Simultaneously the hyaline oocytes turned opaque. An *F*-test showed that the shrinkage was followed by a significant increase in the variance of the hyaline oocytes ($P \ll 0.001$) (the 95% confidence interval became about 50 μm larger) but not for the vitellogenic oocytes ($P > 0.50$).

Production of the Hyaline Oocytes

Prior to spawning, a batch of hyaline oocytes is formed (Fig. 2). To study the production of this batch in greater detail, the elapsed part of the spawning interval (how long the fish was in the spawning interval when killed), was first calculated using the following formula: (time interval between killing and last spawning/predicted spawning interval)·100 (%) (Table 1). A spawning interval of 60 h was set for the indoor spawning (at 8°C) while for Female 1, the only outdoor female used in the present study, the time between batch 1–2 (at 5.5°C) was assumed to be 99 h (cf. Table III in Kjesbu 1989). The egg counter indicated the number of different types of oocytes in each ovary including those destined to be spawned in the next batch (Table 1). The bimodal distribution of oocytes in an advanced spawning interval results in the formation of final growth oocytes (Fig. 2c). In Table 1 the number of oocytes expected to be shed in the next batch is also given. For unstressed fish the number of eggs in each batch follows a dome-shaped curve, for stressed fish this is not the case (Kjesbu 1989).

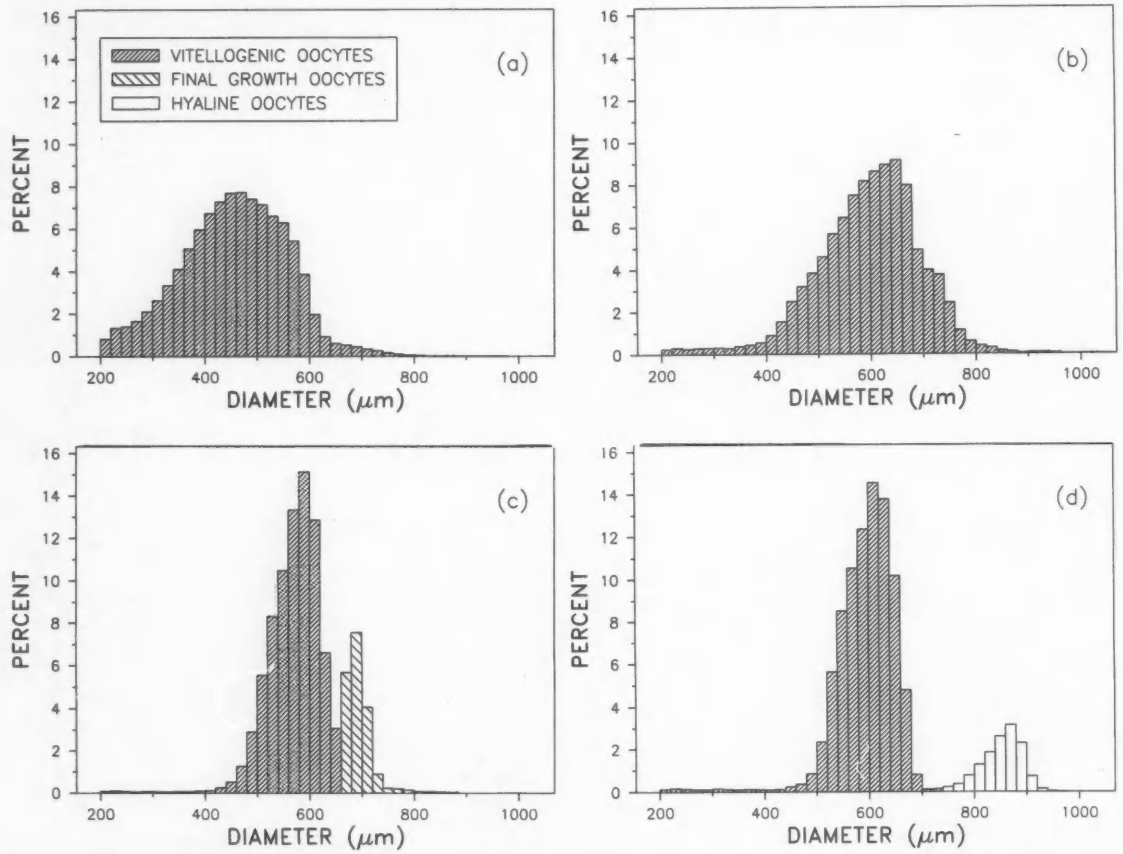

FIG. 2. Oocyte size frequency distribution in four reared females. (a) Prior to spawning, Female E. (b) 8 h after batch 6, Female 4. (c) 13 h after batch 10, Female 6. (d) 31 h after batch 8, Female 5.

TABLE 1. Estimates of the number of oocytes assigned to the next batch and the elapsed part of the spawning interval.

Female	Number of batches shed	Time interval between killing and last spawning (h)	Elapsed part of spawning interval (%)	Number of oocytes prepared for the next batch (thousands)				
				Final growth oocytes	Hyaline oocytes	Ovulated oocytes	Total number	Expected[a] number
E	0							
F	0							
1	1[b]	31	31	210	7		217	200
2	2	37	62		120		120	125
3	4	41	68		170		170	175
4	6	8	13		5		5	250
5	8	31	52		140		140	175
6	10	13	22	280	4		284	275
7	12	9	15		42		42	225
8	16	7	—[c]		120	105	225	125
13	4	29	—		220	215	435	—
14	5	56	—	290	30	390	710	—
16	6	1	—		25	175	200	—
18	8	28	—		10	460	470	—

[a]Found by extrapolation, assuming a dome-shaped curve (Kjesbu 1989).
[b]Outdoor spawning.
[c]Unknown.

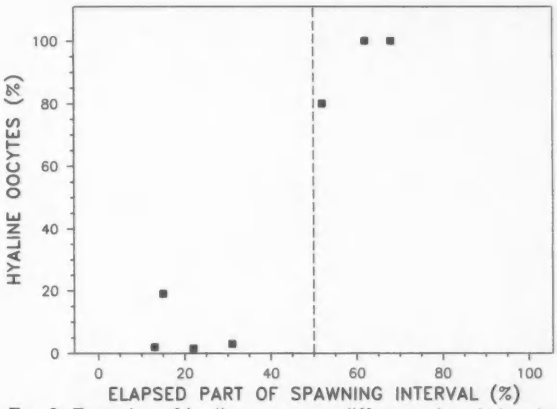

FIG. 3. Formation of hyaline oocytes at different points during the interval between batches. The percentage of hyaline oocytes refers to the expected number of eggs to be shed in the next batch. The situation to left of the broken line represent "few hyaline oocytes present," to right "many hyaline oocytes present" corresponding to over and under 50% of elapsed time to spawning, respectively.

Consequently, these plots for Females 1–8 were used, and the number of eggs in the next batch was found by extrapolation.

The percentage of hyaline oocytes formed was calculated for Females 1–7 from the ratio: number of hyaline oocytes present/expected number of oocytes to be shed in the next batch. For Female 8, originally a female defined as unstressed, the presence of many ovulated oocytes a few hours after spawning indicated that the fertilization rate in the subsequent batch was going to fall considerably (Kjesbu 1989), this specimen was therefore now classified as stressed. If the percentage of hyaline oocytes formed was >95%, the hydration process was assumed to be completed and the percentage was set to 100%. The obtained values were plotted against the elapsed part of the spawning interval (Fig. 3). A baseline was introduced to divide the figure into two parts: "few hyaline oocytes present" and "many hyaline oocytes present," or, over and under 50% of elapsed time to spawning, respectively.

Changes in Characteristics of the Vitellogenic Oocytes

Introduction of the term PES

The eggs shed by Females 1–8 (Table 2) floated (i.e. healthy fertilized eggs) and consequently the loss of eggs due to settling was assumed to be negligible (cf. Fig. 5 in Kjesbu 1989). The observed fecundity was found by adding the number of oocytes shed (eggs collected in the chambers/tanks) and the number of oocytes still left in the ovary (found by use of the egg counter) (Table 2). In the latter case, the variation among samples (Sample 1,S versus Sample 2,S) were examined: the variation was 1.1 and 3.6% for Females 3 and 7, respectively. (One of the prespawning specimens, Female E, was examined in the same way, the values were identical.)

A term called PES (portion of total number of eggs spawned per season) was introduced and defined as:

$$\text{PES} = (\text{Number of eggs shed/observed fecundity}) \cdot 100\ (\%).$$

The PES was estimated for Females 1–8 based upon the data in Table 2, but the total number of oocytes prepared for the next batch (Table 1) was added to the numerator. In Table 3 the reared females are presented by increasing PES-values. Female 7 has changed position in relation to Table 1 (shed 12 batches of eggs but now situated before Female 5 with its 8 batches). For Females 3 and 7 there was no difference and 1% difference among samples in PES, respectively.

TABLE 2. Observed fecundities of the spawning females. ( ) = Sample 2,S

Female	Fecundity (millions)		
	Number of eggs shed	Number of oocytes left in ovary	Observed fecundity
1	0.10	5.03	5.13
2	0.10	3.17	3.27
3	0.42	3.70	4.12
		(3.66)	(4.08)
4	0.73	3.25	3.98
5	1.03	1.06	2.09
6	2.70	1.44	4.14
7	2.15	1.94	4.09
		(1.87)	(4.02)
8	3.08	1.09	4.17

Introduction of the term NG

The final growth oocytes and the hyaline oocytes were removed from the oocyte size distribution and the number of vitellogenic oocytes present was calculated. This number was divided by the ovarian weight giving the number of vitellogenic oocytes per gram ovary, abbreviated NG.

Relationship between PES and NG

The values obtained for NG are given in Table 3. Those for the wild specimens are roughly; rounded off to the nearest 500 (the others to the nearest 10). NG in relation to PES is illustrated in Fig. 4, the data for Females 1–8 were used and the ovaries were separated into those showing many (Females 2, 3, 5, and 8) and those showing few hyaline oocytes (Females 1, 4, 6, and 7) (cf. Table 1, Fig. 3). The change in NG observed for the spawning fish was not correlated logically (i.e. r positive; $r=0.989$, $P<0.01$) or significantly ($r=-0.659$, $P>0.05$), respectively, with mean vitellogenic oocyte diameter. Consequently, the observed change was attributed mainly to change in ovarian consistency. The NG value increased when PES approached zero, supported by the data for Female 1, 2, the two prespawning fish (Table 3), and unpublished data on wild prespawning fish (O. S. Kjesbu). The relationship was exactly linear ($r^2=1.0$) for both conditions when PES exceeded about 10%, the regressions, however, were based only on three points:

$$\text{NG} = 3795 - 25.3 \cdot \text{PES (many hyaline oocytes)}$$

$$\text{NG} = 4480 - 24.8 \cdot \text{PES (few hyaline oocytes).}$$

GM regression gives the most accurate regression line when there are few observations and both X and Y are associated with "errors" (Ricker 1973), but when $r=1$ simple linear regression equals GM regression (Sokal and Rohlf 1981). A comparison of the data from the two conditions (ANCOVA), showed a significant difference in the intercepts ($P \ll 0.001$) but not in the slopes ($0.10<P<0.25$).

As the purpose of the present paper was to make it possible to find the PES-value of fish of unknown history, the two equations were solved for PES giving, for the condition of many and few hyaline oocytes, respectively:

$$(1)\quad \text{PES} = 150 - 0.04 \cdot \text{NG} \quad (\text{NG} < \approx 3750)$$

TABLE 3. Portion of the total number of eggs spawned (PES), number of vitellogenic oocytes per gram ovary (NG) and descriptive statistics of the vitellogenic oocyte mode. The oocytes have experienced the same degree of shrinkage. ( ) = Sample 2,S

Female	Total length (cm)	Egg diameter of preceding batch (mm)	PES (%)	NG	Mean diameter (μm)	SD (μm)	Skewness	Kurtosis
				Reared specimens				
E	69	—	0	12780	418	95.9	0.22	0.43
				(12780)	(402)	(97.7)	(0.41)	(0.79)
F	66	—	0	9640	456	101.3	−0.03	0.11
1	62	1.37	6	5730	448	124.2	−0.18	−1.06
2	69	1.50	7	3890	584	124.8	−0.68	−0.01
3	72	1.49	14	3440	572	112.2	−0.42	−0.60
			(14)	(3400)	(579)	(111.4)	(−0.45)	(−0.52)
4	69	1.37	18	4030	578	97.8	−0.59	0.96
7	61	1.35	54	3150	551	83.3	−0. 53	−0.08
			(55)	(2990)	(552)	(85.1)	(−0.51)	(−0.11)
5	61	1.34	56	2380	551	54.4	−1.70	7.11
6	65	1.31	72	2690	521	47.9	−1.22	4.30
8	68	1.28	80	1770	545	92.5	−1.45	2.76
				Wild specimens				
W1	80	—	—	≈2500	544	86.9	−0.82	1.16
W2	101	—	—	≈3000	498	107.6	−0.38	−0.35
W3	85	—	—	≈1000	573	98.4	−2.03	3.70

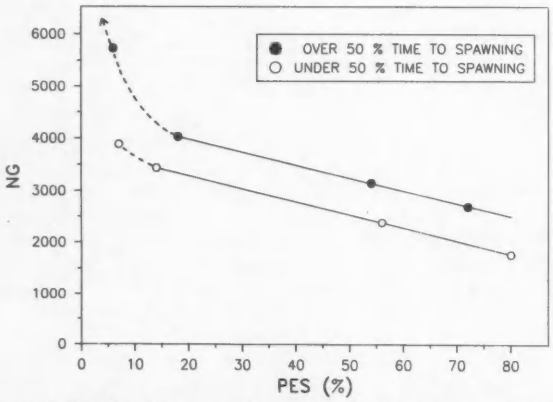

FIG. 4. Relationship between the number of oocytes per gram ovary (NG) and the portion of number of eggs spawned per season (PES). The meaning of the legend is given in Fig. 3. The broken lines were drawn by hand.

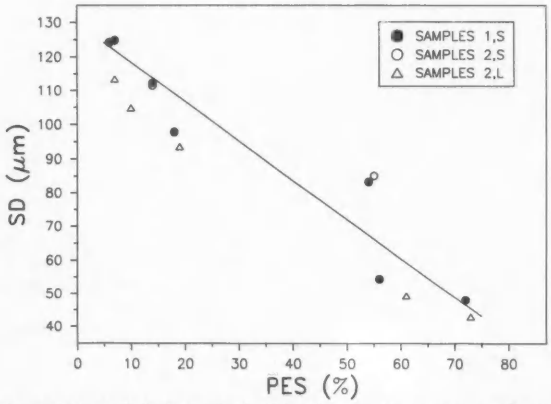

FIG. 5. Standard deviation (SD) of the vitellogenic oocytes as a function of portion of the total number of eggs spawned per season (PES). S refers to short time in 2% formaldehyde, L to long. The regression line was estimated for Samples 1,S.

(2) $PES = 180 - 0.04 \cdot NG$ ($NG < \approx 4500$).

Vitellogenic oocyte mode

Statistics describing the vitellogenic oocyte mode were calculated for all females (Table 3). A correction was made for the varying period the samples stayed in Gilson's fluid, because of the continuous shrinkage of the oocytes (Fig. 1). The two prespawning specimens stayed in the fluid for 172 d, the spawning specimens for 5 to 58 d less. It was assumed that the shrinkage caused the whole mode to be displaced to the left. The maximum stay in Gilson's fluid was chosen as a base value; thus, only the modes of the spawning specimens needed to be displaced. The shrinkage recorded for vitellogenic oocytes from Females 2 and 5 (period: Day 1 onwards) was studied in more detail. An ANCOVA-test showed that the shrinkages (i.e. the slopes) were clearly not significantly different ($P > 0.75$). The shrinkage was 0.142 and 0.146%·d^{-1}, respectively, the mean was used in the corrections. It was necessary to displace the modes by 4–48 μm.

Relationship between PES and SD

There are always some hyaline oocytes present in the ovaries of spawning specimens (Fig. 3). Consequently if no hyaline oocytes are seen during macroscopial examination, it can be concluded that PES = 0.

The standard deviation of the vitellogenic oocyte mode (SD) of the reared spawning specimens decreased with increasing PES (Table 3). There was, however, a sudden increase for the last female. This phenomenon was assumed to be caused by the rather consistent diameter range (Fig. 2); there is a turning point in the SD when the number of oocytes in the middle region of the mode becomes low and the relative contribution of the tails to the SD therefore increases. The relationship between SD

and PES, using Females 1–7 (Fig. 5) and GM regression, was described by the following equation:

$$SD = 130 - 1.16 \cdot PES \ (r^2 = 0.917, P \ll 0.01, 5\% < PES < 75\%)$$

The variation among Samples 1,S and Samples 2,S was considered insignificant in relation to the variation around the regression line. Solving the equation for PES gives:

$$(3) \quad PES = 112 - 0.86 \cdot SD \ (NG > 2000).$$

Equation (3) can not be used when the fish is close to a spent condition explaining the assumption NG>2000 (Table 3). The absolute residuals, |observed PES − predicted PES|, were calculated: the mean was 5.5% (95% confidence interval: 0.6–10.4%). The residual of Female 7 was clearly the highest: 13.6%.

A standard statistical test, as Student *t*-test or Kolmogorov-Smirnov two-sample test, for comparison of size frequency distributions was found to be over-sensitive when the number of observations was extremely high as in the present paper where number of oocytes in sample was >25 000. To reveal the effect of varying periods in the 2% formaldehyde solution, it was therefore decided to focus on SD and Equation (3). Standard deviation and PES based on Samples 2,L (Females 1, 2, 4, 5, and 6) were included in Fig. 5. PES was about 2% higher (range: 0–5%) in Samples 2,L than in Samples 1,S. The SD was about 10% lower (range: 4–16%). The number of oocytes recorded was about 4% lower (0.4–8%: 15 000–275 000 oocytes) explaining the increase in PES. No oocytes were noticed below 300 μm in the long period samples while the short period samples (1,S and 2,S) showed oocytes all the way down to 200 μm. In the range 200–300 μm, Samples 1,S showed not more than 5000–85 000 oocytes (0.2–2%) indicating for Samples 2,L that some larger oocytes must have been lost to account for the 4% drop in total oocytes. When the SD (y) versus PES (x) regression based on Sample 2,L was compared with that from Sample 1,S, using ANCOVA, neither the intercepts ($0.05 < P < 0.10$) nor the slopes were significantly different ($P > 0.75$). Nevertheless, there was one important aspect of the reduction in SD (mean: 9 μm): if samples are treated in the same way as Samples 2,L but Equation (3) is used, PES is overestimated by about 8%. Accordingly, the equations given in the present paper should only be used with oocytes that have been subject to the same conditions as the short period samples.

Skewness, kurtosis, mean vitellogenic oocyte diameter, and PES

In relation to PES, both skewness and kurtosis increased abruptly when PES passed a certain limit (Table 3): skewness < -1.0 and kurtosis >2.5 are equivalent to PES $> \approx 55\%$.

The increase in mean vitellogenic oocyte diameter from Females E, F, and 1 to Female 2 was considered to be simply due to oocyte growth. It was further assumed that the value recorded for Female 2 represented maximum diameter, i.e. arrested oocyte growth. The somewhat high value for Female 8 was believed to be a result of the aforementioned turning point. For Females 2–7 mean vitellogenic oocyte diameter was significantly correlated with PES; $r = -0.958$ ($P < 0.01$).

In some instances (Kjesbu 1989) egg diameter has been shown to decrease by about 0.5% from one batch to the next. However this is not a general phenomenon and is disregarded in the present work; the egg diameter of the subsequent batch was set equal to that of the preceding batch. To investigate the relation of the mean vitellogenic oocyte diameter to the subsequent egg diameter, a correlation was developed using the data for Females 1–8 (Table 3). Subsequent egg diameter and mean vitellogenic oocyte diameter were not significantly cor-

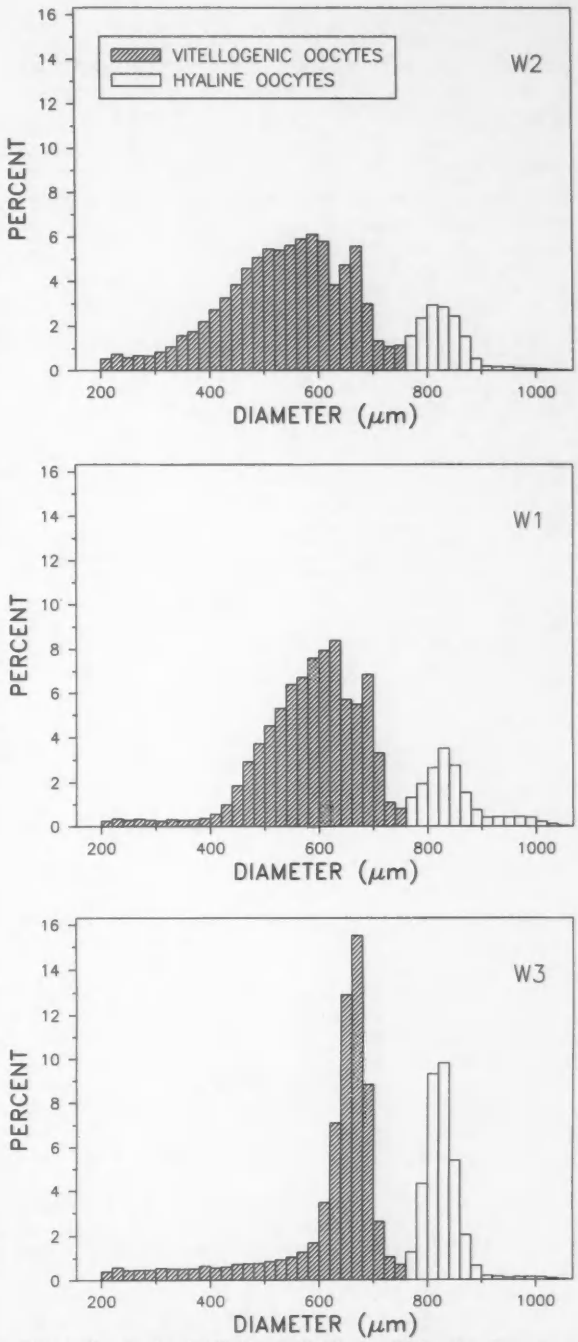

FIG. 6. Oocyte size distribution in the three wild females. From top to bottom, the panels are arrayed in increasing PES-values. W2: 101 cm, 9 yr; W1: 80 cm, 6 yr; W3: 85 cm, 6 yr.

related ($r = 0.392$, $P \gg 0.05$). The latter was then subtracted from the former and the difference obtained correlated with the corresponding PES: the correlation was clearly significant ($r = -0.863$, $P < 0.01$).

Application of PES as an Indicator of Stage of Spawning

The three wild spawning females (Fig. 6) caught off Lofoten were classified as Norwegian Coastal cod. Two showed an apparent trimodal distribution, but since many hyaline oocytes were present, it was assumed by comparing them with the distribution in Fig. 2c that final growth oocytes had not started to bud off. The vitellogenic oocyte mode of two of the reared females (Females 1 and 3) were not smooth either, so this was not considered to be specific to wild females.

The volume displacement method used here to estimate ovarian sample weight makes it difficult to rely completely on the results from equation (1) for W1 and W2: PES = 50 and 30%, respectively. It seems, however, to be clear that W3 has a PES > 80%. This is because of the very low NG (cf. Table 3). In addition, its skewness and kurtosis show that it has at least spawned more than half of its eggs. Equation (3) gives a PES of 37% for W1 and of 19% for W2. The mean vitellogenic oocyte diameter recorded must be seen in the light of the corresponding NG to be of any help: the diameter in W1 suggests a medium PES, that of W2 a low PES (i.e. growing oocytes) and that of W3 a high PES (i.e. oocytes after the turning point).

Taking into consideration the above suggested range for the PES-residuals and the lack of reliable data on ovarian sample weights, it was decided to designate four stages of spawning: (1) PES ≤ 25%: early; (2) 25% < PES ≤ 50%: early/middle; (3) 50% < PES ≤ 75%: middle/late, and (4) PES > 75%: late. Thus, W1, W2, and W3 represent the stages 2, 1, and 4, respectively.

Discussion

A normal ovulatory rhythm of cod is assumed to be as follows: (1) just after spawning, only vitellogenic oocytes and a few remnant hyaline oocytes from the previous batch(es) are present. (2) When about 20% of the spawning interval has elapsed, a mode of final growth oocytes bud off. (3) At about 50% of the spawning interval, the final growth oocytes are rapidly hydrated, forming the hyaline oocytes. (4) The hyaline oocytes are finally expelled from the follicle by active contraction of the follicle (Goetz 1983). (5) The ovulated oocytes are retained in the ovarian lumen for only a few hours before they are shed (McEvoy 1984). Accordingly, assuming a spawning interval of 60 h, oocyte growth is slow during the first 12 h (an "arrest"), the final growth oocytes are formed during the next 18 h and the hyaline oocytes are numerous during the following 25 h. Finally, the ovulated oocytes are kept in the ovarian lumen for approximately 5 h before spawning.

The formation of the final growth oocytes is of particular interest because of the lack of correlation between mean vitellogenic oocyte diameter and subsequent egg diameter indicating that egg size is determined during the final growth period (egg diameter and egg dry weight have been shown to be very closely correlated (Kjesbu 1989)). The present work indicates that the difference between the mean vitellogenic oocyte diameter and the subsequent egg diameter diminishes as PES (portion of total eggs spawned/season) increases. This may be caused by depleted yolk reserves (cf. Bromley et al. 1986).

There is strong evidence (Fig. 5) that a change in PES is reflected in the shape of the vitellogenic oocyte mode — the standard deviation decreases as PES increases. Also, the range of oocyte diameters remained constant during the spawning period but there were changes in dominance of the various developmental stages (Sorokin 1957; Kjesbu and Kryvi 1989). The present study shows that to obtain PES from NG, ovaries containing many and few hyaline oocytes must be treated separately (Fig. 4). It is also necessary to get accurate sample weights. In field conditions this is often difficult; it is recommended that the tare is recorded before the cruise and that the bottle is reweighed back in the laboratory. There are further possible limitations in the application of equations (1) and (2) to different stocks. Unpublished results (O. S. Kjesbu) on Northeast Arctic cod suggest that the NG in prespawning ovaries of this stock is significantly lower; the same may be the case for spawning fish. However, equation (3) which relates the portion of eggs spawned to the standard deviation of the oocyte distribution is probably more robust. Nevertheless, the NG–technique is simpler and can be done without the egg counter.

For stock assessment, fecundity has been measured prior to spawning (Hislop and Hall 1974). The proposed method permits estimation of fecundity for fish that have commenced spawning. As cod appear to spawn a constant number of batches at consistent intervals, it is also possible to investigate residence on the spawning grounds. Individual batches of oocytes are known to differ in fatty acid profiles (Ulvund and Grahl-Nielsen 1988) and oocyte number and size (Kjesbu 1989). Therefore it is important to identify individual batches when relating parental characteristics such as age or condition to larval survival. The method can also be used analogously in endocrinology. Greeley et al. (1988) have recently shown a pronounced peak in 17β-oestradiol and consequently in vitellogenin production in *Fundulus grandis* between batches, i.e. probably during the final growth period. The same seems to be the case in halibut (*Hippoglossus hippoglossus*) (Dr. Birgitta Norberg, Austevoll Marine Aquaculture Station, N-5392 Storebø, Norway, unpubl. data).

This method, calibrated for individual stocks, enables potential fecundity estimates to be made on spawning rather than prespawning fish. With a knowledge of time intervals between batches useful information could be derived about residence times on spawning grounds. The ability to identify individual batches which may have different characteristics has relevance to hormonal analyses and to the relation between parental characteristics and larval survival.

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Size-Structured Mortality in a Threespine Stickleback (*Gasterosteus aculeatus*) – Cutthroat Trout (*Oncorhynchus clarki*) Community

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Reimchen, T. E. 1990. Size-structured mortality in a threespine stickleback (*Gasterosteus aculeatus*) – cutthroat trout (*Oncorhynchus clarki*) community. Can. J. Fish. Aquat. Sci. 47: 1194–1205.

In a 112-ha bog lake on the Queen Charlotte Islands, British Columbia, small fish comprised the major element in the diet of cutthroat trout (*Oncorhynchus clarki*). Despite the presence of juvenile salmon and char in the lake, threespine stickleback (*Gasterosteus aculeatus*) was the most common fish in the diet (99.5%). Foraging activity appeared to be more frequent in littoral than in limnetic regions. Mark–recapture methods indicate an average population of 220 trout and 75 000 adult stickleback. Trout consumed an estimated 308 770 stickleback yearly (145 kg) of which 65% were taken during summer. Seventy-three percent of all fish consumed were young of the year and 2% were adults, the latter representing 4% of the adult population in the lake. There was a 75% reduction in total mortality between successive year classes of stickleback (0,1,2,3+). This consumption curve, which resembles a typical survivorship curve of fish, is a function of the size-structure of the populations and includes interactions between size availability of stickleback, prey-size preferences of the trout, and length frequency distributions of trout. Total weight of stickleback consumed by trout comprised about 40% of that previously calculated for 16 species of avian piscivores in the lake.

Dans un lac-tourbière de 112 ha des îles Reine-Charlotte (Colombie-Britannique), les petits poissons constituent l'élément principal du régime alimentaire de la truite fardée (*Oncorhynchus clarki*). Malgré la présence de salmonidés juvéniles dans le lac, l'épinoche (*Gasterosteus aculeatus*) est la principale proie (99,5%). La recherche de nourriture semblait plus fréquente dans la région littorale que dans la région limnétique. Les résultats d'expériences de marquage et de recapture révèlent une population moyenne de 220 truites et de 75 000 épinoches adultes. La truite consommait environ 308 770 épinoches par an (145 kg) dont 65% étaient capturées à l'été. Soixante-treize pour cent de tous les poissons consommés étaient des nourrains et 2% étaient des adultes qui constituaient 4% de la population adulte peuplant le lac. On a noté une baisse de 75% du taux de mortalité total entre des classes annuelles successives d'épinoches (0, 1, 2, 3+). La courbe de consommation, qui ressemble à une courbe typique de survie du poisson, est une fonction de la structure des tailles chez les populations et comprend des interactions entre la disponibilité de chaque taille d'épinoches, les préférences de la truite quant à la taille de la proie et les distributions de la fréquence des longueurs de la truite. Le poids total des épinoches consommées par la truite représente environ 40% du poids total calculé pour 16 espèces d'oiseaux piscivores fréquentant le lac.

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Integration of natural history, theory, and experimentation have advanced our understanding of the dynamics of predator–prey interactions (for example, Werner et al. 1983; Taylor 1984). Although not discussed in recent evolutionary syntheses (Futuyma 1979; Endler 1986; Feder and Lauder 1986), one substantive deficiency in an otherwise extensive literature on predator–prey interactions are data on sources and extent of mortality throughout the survivorship curve of the prey. Such information is fundamental in assessing interpopulation and ontogenetic differences in prey defenses.

The threespine stickleback (*Gasterosteus aculeatus*) has been used in numerous behavioral and morphological studies over the last 50 yr (for reviews see Wootton 1984; Bell 1984). Differences in phenotypic traits frequently occur between populations and are occasionally associated with presence or absence of predators (Hagen and Gilbertson 1972; Moodie 1972; Moodie and Reimchen 1976; McPhail 1977; Gross 1978; Reimchen 1980; Giles and Huntingford 1984). This species is a logical candidate for studies of sources and extent of age-specific mortality. As part of a long-term investigation of intra-population variability in prey defenses in the threespine stickleback, I undertook such an evaluation in an ecologically undisturbed population. These ongoing studies (Reimchen 1983, 1988, 1990) show a complex of at least 21 species of avian, salmonid, mammalian, and invertebrate piscivores. Fourteen species of avian piscivores consume from 295–573 kg fish·y^{-1}, most of which are probably stickleback (Reimchen and Douglas 1984). In this paper, I focus on the most common predatory fish in the lake, cutthroat trout (*Oncorhynchus clarki*), and quantify diet and age-specific predation of stickleback. To provide a realistic estimate of consumption, data were collected on seasonal differences in (a) foraging rates of the predator, (b) population size and length distribution of predator, (c) dietary proportion of prey items for each length class of predator, and (d) length frequency distributions of the prey eaten by each size-class of the predator.

Methods

Study area

The study was carried out at Drizzle Lake (112 ha, elevation 55 m), Queen Charlotte Islands, British Columbia. Historically,

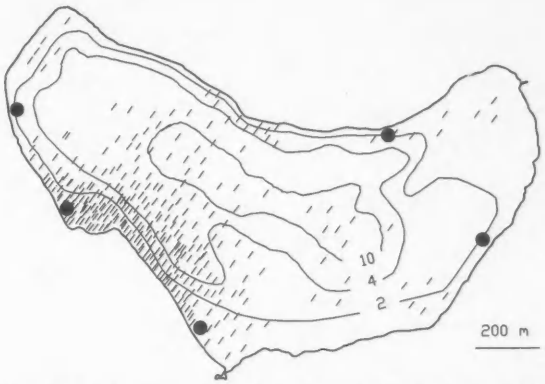

FIG. 1. Bathymetric map of Drizzle Lake showing locations of fyke net (●) and gill net (/). Depth contours in meters.

this remote watershed has received negligible disturbance from human activity and is currently protected under the Ecological Reserves Act (Krajina et al. 1978). General habitat of this watershed has been described (Reimchen and Douglas 1980, 1984; Stinson 1982). The substratum in Drizzle Lake is a combination of coarse sand, gravel, and small boulders (generally less than 15 cm in diameter). Since it is a dystrophic and deeply coloured lake (transmission at 400 nm =67%, Reimchen 1989), aquatic macrophytes are uncommon, apart from several shallow bays where the water lily (*Nuphar luteum*) achieves moderate densities (1 plant/3 m^2). Small submerged plants (*Eleocharis*, *Juncus*, and *Lilaeopsis*) occur near shoreline in low abundance. Foraging cover, such as fallen trees and other submerged debris, is uncommon. Bathymetry is relatively simple with gentle gradients on most of the perimeter except the central northern area where it drops off more steeply (Fig. 1).

There are resident populations of threespine stickleback, cutthroat trout, Dolly Varden (*Salvelinus malma*), and coho fry (*Oncorhynchus kisutch*) in the lake. Sixteen species of avian piscivores have been observed over a 5-yr period (Reimchen and Douglas 1984). River Otter (*Lutra canadensis*) occasionally enter the lake from adjacent streams. The stickleback are characterized by extensive melanism, exceptionally large adult body size (70–110 mm) with stout dorsal and pelvic spines (Reimchen et al. 1985) and are similar to the endemic black stickleback initially described for Mayer Lake (Moodie 1972) which occurs 20 km to the south.

Estimating Population Size and Size Distribution

Ascertaining the actual number of trout in the lake at any single time poses several difficulties. Small trout (<15 cm) enter the lake from inlet streams usually with coho salmon fry in spring. Some move directly into the outlet stream while others remain in the lake. The latter are subject to predation from diving birds. Larger trout also move into the outlet stream for periods up to 6 mo before returning to the lake (judged by the recapture incidence of marked fish). Given these constraints, numbers of cutthroat trout (hereafter, trout) in the lake were estimated with mark–recapture studies from 1983–85 and in 1988. A standard fyke net (hoop diameter 1.5 m, mesh 5 mm) was set primarily in littoral regions of the lake since the previous period of gillnetting (1976–83) indicated most activity close to shore. Those set in limnetic regions rarely yielded fish which is largely a consequence of the poor capture efficiency of this net in open water. Hoops were generally set at 1.5–2 m water depth. A seine net (with additional floats attached to the float line) was attached to the hoop and extended to the shoreline from the edge of the trap entrance. The fyke net was left undisturbed generally for 3–4 d (range 1–7) and then raised and the fish removed and counted. Trout were measured for standard length, marked and released. The net was then reset at a different site on the lake (Fig. 1). Throughout the study, marked fish were often recaptured within 3 d of their release on the opposite side of the lake indicating extensive movement and shoreline dispersal. I gill-netted in the central parts of the lake on several occasions during the marking program to determine whether there was dispersal of marked fish from littoral into limnetic regions; of three fish netted, two were recaptures, indicating mixing of inshore and offshore trout. Total collecting time with the Fyke net was 148 d in 1983, 246 d in 1984, 173 d in 1985, and 80 d in 1988.

For most marking techniques, I used a nylon line with colored beads placed through the dorsal musculature at the insertion of the dorsal fin. During 1983–85, the color of the beads was changed every 2–3 mo on unmarked fish while in 1988, trout were marked with individually identifiable tags. During the first year of marking, about one-third of the tags were lost. In these cases, I was still able to identify a general recapture by the two small holes or healed skin at the insertion site although I was not able to place the fish within any specific marked group. The marking procedure was modified by producing a loop to the nylon line which was knotted and fused. Tag loss was largely eliminated (<5%). Several methods were used for estimating population size. For most purposes, I employ a sequential series of independent Petersen's estimates (based on monthly mark–recapture data) to arrive at a best fit curve of population numbers over the year (equations 3.7 and 3.8, Ricker 1958). These estimates are compared with those derived using Schnabel and Jolly-Seber methods (Seber 1982).

Detailed methodology for estimating population size of stickleback in the lake will be presented separately but a brief description follows here. In spring 1985, 17 033 adult stickleback (SL 70–95 mm) were marked and released throughout the lake, of which 3803 were recaptured in summer. Based on both Petersen's and Schnabel's methods, population estimates of adult stickleback ranged from 30 000 to 120 000 ($\bar{x}$ = 75 000). Nest densities and numbers of breeding males in different littoral areas were recorded; I estimated there to be 10 000–60 000 nests over the 3-mo breeding season with an average of 395 eggs per nest (range 166–1014, N = 32). Assuming the occurrence of 30 000 nests and complete hatch, recruitment would be 12 million fry. Because of egg consumption by some adult stickleback (16.4%) and cannibalism of fry by a low percentage of adults (3%), sub-adults (1.5%) and juveniles (0.2%), as well as whole-nest predation by Dolly Varden (Reimchen 1990), a substantial but unknown proportion of the 12 million potential recruitment will be lost.

Evaluating Diet

Trout (>10 cm) were captured with a variety of monofilament gill nets (10–100 mm stretch mesh diameter, usually 20 m total length) set in a diversity of localities throughout the lake from 1976 to 1983 (Fig. 1). I did not net during periods of extensive lake occupancy by diving birds in mid-summer or

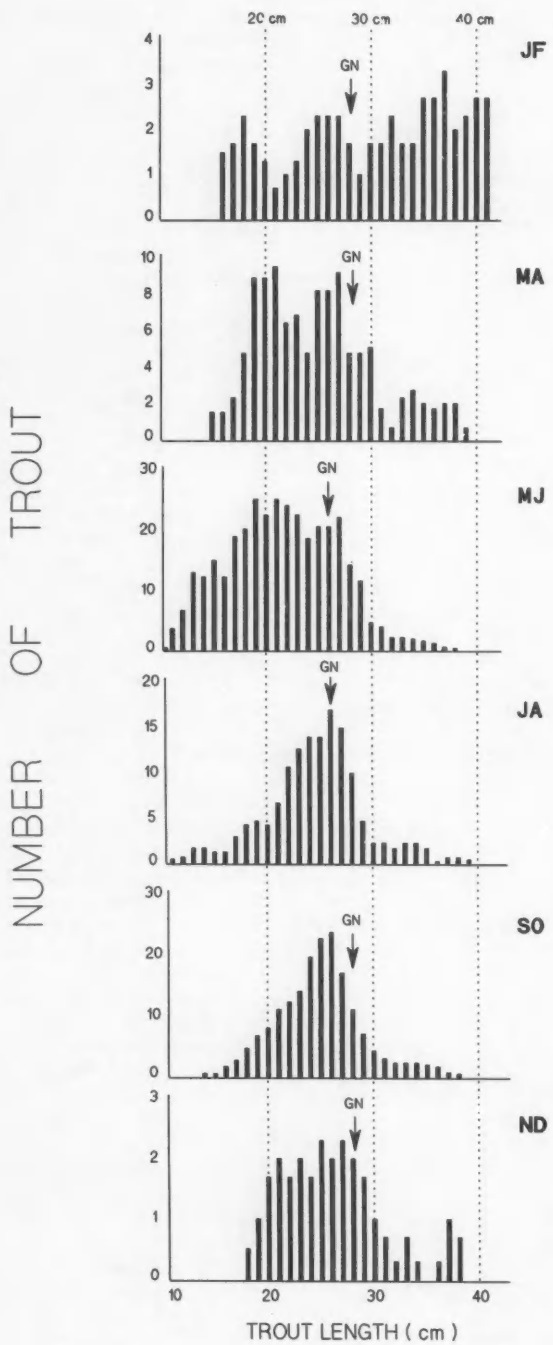

FIG. 2. Standard length of trout captured in fyke net at bimonthly intervals (1983–85). Histogram shows three-point moving average for 1-cm length-groups in raw data. GN = mean size of trout captured in gill nets (1976–83).

during ice cover which occurred irregularly from November to March. Because numbers of trout in the lake were unknown, I generally limited the gill-netting period to obtain, on average, a single trout per week throughout the year suspecting that this was a very conservative exploitation rate for a 112-ha lake. As population estimates of trout were later to demonstrate, even this exploitation rate was high.

Gill nets were generally checked near dawn and again several hours prior to dusk. Standard length of trout was recorded to the nearest centimetre and the stomach contents preserved in formaldehyde. Prey in the stomachs were identified (invertebrates to order, fish to species where possible) and numbers of each fish prey determined. I made visual estimates of the relative volume of the major taxa in the stomachs. Beginning in autumn 1979, intact fish prey were measured for standard length. Undigested stickleback spines, occasionally with tissues attached, were common in the pyloric region of the stomach; I measured length of these spines under a dissecting scope equipped with an ocular micrometer. Left and right pelvic spines were differentiated to allow determination of numbers of stickleback eaten per fish. Spine length and body length were determined on a sample of living stickleback to establish the regression equation from which original body sizes of stickleback in the trout stomachs were reconstructed.

Estimating Total food Consumption

Water temperature and body weight are the two major variables in calculating optimal daily food rations for trout (Elliott and Persson 1978). Accordingly, lake temperature was recorded (100 m from shore, 0.1 m below surface, 1200 h) monthly for 2 yr (1984, 1985). I tabulated the length distribution of trout collected in the fyke net during bimonthly periods (1983–85). Based on the monthly population estimates of trout, I calculated for each 1-cm length class the predicted numbers of trout in the lake of this size-class. To obtain a conversion from length to weight (required for estimating daily food requirements), I weighed 124 trout to the nearest 10 g over a range of standard lengths (120–400 mm) during the 1988 marking program. This yielded the least squares regression:

$$[1] \quad W = 0.000007821 \cdot L^{3.0626},$$

where W = weight of trout (g) and L = standard length of trout (mm) (intercept = -11.76, $r^2 = 0.95$, $p < 0.001$). Weights were estimated for each 1-cm length-class of trout.

A length-weight relationship was determined for stickleback (size range 12–90 mm). Moist individuals ($N = 102$) were measured for standard length and weighed ($\pm$ 0.001 g) on an electronic balance. This produced the regression:

$$[2] \quad W = 0.000006803 \cdot L^{3.121},$$

where W = weight (g) and L = length (mm) (intercept = -11.9, $r^2 = 0.99$, $p < 0.001$). I used the following length and weight groups: 7–9 mm = 0.005 g, 10–14 mm = 0.016 g, 15–19 mm = 0.047 g, 20–29 mm = 0.157 g, 30–39 mm = 0.45 g, 40–49 mm = 0.98 g, 50–59 mm = 1.84 g, 60–69 mm = 3.09 g, 70–79 mm = 4.83 g, > = 80 mm = 7.14 g.

These and additional equations (see below) were combined with all variables (Table 3) into a general computer program to determine monthly stickleback consumption (weight, numbers, and size-specific numbers).

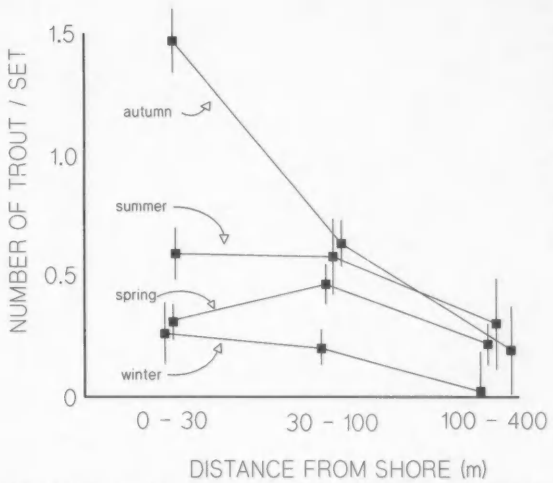

FIG. 3. Gill net effort (mean number of trout per set) at Drizzle Lake (1979–83) in relation to lake position and season. Number of sets in each lake position are 229, 290, and 137, respectively. Number of sets for winter, spring, summer, and autumn are 168, 254, 111, and 123, respectively.

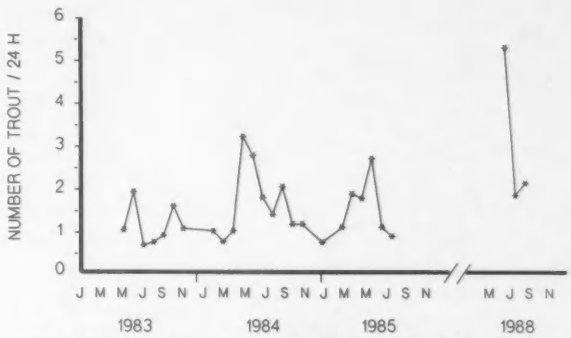

FIG. 4. Number of trout captured in fyke net/24 h. Each data point comprises cumulative effort for 15–31 d/mo.

Results

Population Characteristics of Trout and Stickleback

Standard length of trout captured in the fyke net ranged from 10–41 cm with a mode at 22 cm. Bimonthly frequencies of length-classes differed over the year (Fig. 2), with proportionally more large trout (>30 cm) occurring in January–February and more small trout (10–20 cm) in May–June. From July to December, most trout were from 20–30 cm. Very small trout (<10 cm) were not present in the lake but these were observed in confluent streams.

Capture rate in gill nets (number per 12 h set) varied seasonally and spatially (Fig. 3). It was lowest in winter (0.20), increasing during spring and summer reaching the highest (0.80) in autumn. This probably reflects seasonal differences in amount of movement and relative abundance. Capture rate was greatest (0.55) close to shore and lowest (0.21) in the central areas of the lake (>4 m depth), the differences being most pronounced during autumn. Two-factor ANCOVA (season and lake position on capture rate with year as a covariate) indicates significant associations (season $F = 29.6_{DF=3}$, $P > 0.001$, lake position, $F = 9.6_{DF=2}$, $P < 0.002$, interaction, $F = 22.6_{DF=6}$, $P < 0.001$, covariate, $F = 1.5_{DF=1}$, $P = 0.2$).

Numbers of trout captured in the fyke net also varied seasonally and yearly. Catches during late spring and early summer were approximately twice that of winter (Fig. 4). The peak in spring results from increased movement of trout during rising temperatures and the influx of small trout into the lake from surrounding creeks. In contrast, there is a sharp reduction during June which may result from off-shore movement, emigration into adjoining streams, and possible mortality from avian piscivores which are present during this period (Reimchen and Douglas 1984). Summer catch was lowest in 1983 and highest in 1988, indicating recovery of population numbers from the earlier gill-netting period.

Yearly population size of trout was estimated with three methods (Table 1). Average population size overall was 198 individuals (Schnabel), 207 (Petersen), and 336 (Jolly-Seber) and with each method, numbers were lowest in 1983 and 1984 following the previous period of gill-netting. Comparisons of estimates in 1988 with those in 1985 yield contradictory trends in population recovery with Petersen producing a reduction in 1988 and Jolly-Seber a population doubling. I consider Petersen's estimates to be most reliable for this investigation. Each estimate is independent of each other and is based on narrow time blocks. As such, these require the fewest assumptions and would be least affected by seasonal differences in mortality, immigration, and emigration.

Seasonal abundance of trout, determined from a series of Petersen's estimates over the year, show lowest numbers in winter and highest in spring and summer (Table 2). Based on a visually smooth-fitted curve, I use the following estimates of trout population sizes in subsequent calculations: Dec./Jan./Feb./Mar. = 115, April = 110, May = 200, June/July = 250, Aug. = 200, Sept. = 100, Oct./Nov. = 90.

Stickleback ranged in size from 7 mm (newly hatched) to 110 mm. Following hatching (from May to August), stickleback achieved a length of 40 mm within 1 yr (0+), 60 mm within 2 yr (1+), 70 mm within 3 yr (2+), and 80 mm within 4 yr (3+). Mean adult size in the population was 80 mm. Individuals can survive for 6–7 yr (T. E. Reimchen, unpubl. data) and consequently the adult cohort was composed of multiple year-classes.

Stomach Analyses of Trout

Overall, 21% of the trout had empty stomachs, most of these occurring from January to April. Stomachs in these winter fish were highly contracted, indicating a lack of foraging, while the occasional summer-captured trout without food had distended stomachs, characteristic of recent digestion rather than a cessation of feeding. Stickleback and invertebrates (primarily winged ants, Hymenoptera, Formicidae) were the dominant food throughout the study period (Fig. 5). Excluding fish with empty stomachs, stickleback occurred in 89.3% of the trout while invertebrates occurred in 21.7%. A comparable pattern occurs with relative volume as stickleback were the major (>80% stomach volume) prey in 85% of the stomachs. Of 1900 fish extracted from the stomachs, 1891 (99.5%) were stickleback and eight were juvenile salmonids. Stickleback were virtually the only prey taken during autumn and winter, but this proportion declined marginally during summer when winged ants comprised the major alternate prey. During this 2-wk

TABLE 1. Population estimates of cutthroat trout at Drizzle Lake. Data from 1988 represents summer only.

Method	1983	1984	1985	1988
Petersen	152(85–260)	128(79–187)	322(197–553)	225(58–563)
Schnabel	160(147–175)	201(178–297)	210(168–270)	220(198–256)
Jolly-Seber		122(48–172)	296(243–348)	589(186–1038)

TABLE 2. Petersen's estimates of cutthroat trout population size at Drizzle Lake. Tagging mark changed for each date.

Number marked (date)	Number caught (date)	Number recaptured	Population size	95% Confidence limits	
48 (5–6/83)	64 (6–8/83)	11	260	130	390
35 (8–9/83)	82 (10–11/83)	23	121	80	162
22 (10/83)	30 (11/83)	7	85	36	134
14 (11/83)	61 (11/83–02/84)	5	145	41	249
66 (2–4/84)	87 (5–7/84)	30	187	134	241
36 (5–6/84	73 (6–7/84)	19	133	84	183
25 (8–9/84)	24 (9–84)	4	125	34	216
40 (9–10/84)	75 (10/84–3/85)	25	117	80	153
11 (11/84)	85 (1–4/85)	11	79	38	119
21 (1–3/85)	86 (4–5/85)	5	305	82	527
20 (4/85)	82 (5–6/85)	2	553	10	1097
35 (5/85)	85 (6/85)	12	232	118	346
35 (6/85)	44 (7–8/85)	7	197	78	316
51 (1/6/88)	60 (3/6/88)	12	239	126	353
	39 (4/6/88)	10	185	94	277
	36 (2/7/88)	11	157	86	229
	22 (4/7/88)	4	235	65	404
	20 (1/8/88)	5	179	64	293
	21 (3/8/88)	3	281	54	507
44 (3/6/88)	39 (4/6/88)	3	440	67	813
	36 (2/7/88)	6	233	84	381
	63 (8/88)	4	563	122	1005
20 (4/6/88)	36 (2/7/88)	3	185	29	341
	63 (8/88)	5	213	60	367
11 (2/7/88)	22 (4/7/88	2	84	6	163
	41 (10/8/88)	7	58	23	92
13 (4/7/88)	21 (3/8/88)	2	95	7	184

period, large rafts of ants floated on the lake following a major annual dispersal from surrounding forests.

Diet was largely independent of size of trout except during summer when small trout had proportionately fewer stickleback in the diet than did larger trout (Fig. 6). Log-linear analyses shows that both size and season contribute unique effects to trout diet (season·diet, length·diet; $P<0.001$ for both interactions).

Number of stickleback per trout stomach ranged from 0 to 55 with a mode at one and a mean of 5.8 (Fig. 7). Bimonthly trends indicate lowest number in spring, increasing values over the summer and highest means in late autumn ($P<0.001$, Kruskal-Wallace test).

Trout consumed stickleback 9–90 mm in length. Size frequency distributions of stickleback eaten differed among seasons (Fig. 8A) and appeared to reflect seasonal variation in size distribution of stickleback available. From September to April, the greatest predation occurred on the 20–40–mm size cohort and there was low but equivalent consumption among each of the larger size-classes. In May/June, there was a broadening of the distribution with similar levels of predation throughout the 20–80 mm range but a moderate mode from 50 to 60 mm. In July/August, distribution was distinctly bimodal, the lower mode (14 mm) comprising young-of-the-year (YOY) recruitment into the population and the second mode (48 mm) corresponding to 1+ year-classes.

Small trout (10–19 cm) took 10–30-mm stickleback with a mode near 20 mm (Fig. 8B). Among progressively larger size-groups of trout, there was a graded increase in average size of stickleback such that among trout >35 cm, modal length of stickleback in the stomachs was about 70 mm. Size relationships between predator and prey were similar during each bimonthly interval although the strength of the relationship is variable (Fig. 9). Correlation coefficients were lowest in summer and highest in autumn and winter. Variability of prey sizes was also associated with predator size since larger trout took a greater size range than did small trout ($P<0.001$, for each bimonthly period, Bartlett's test for homogeneity).

Estimating Total Prey Consumption for the Trout Population

Daily food requirements of trout

Estimates of optimal daily food consumption (maintenance and growth) of salmonids were derived from the following equation (Elliott 1976):

$$[3] \quad Dcal = 15.116 \cdot W^{0.767} \cdot e^{0.138 \cdot T},$$

where $Dcal$ = daily consumption in calories, W = weight of trout (g), and T = water temperature (°C).

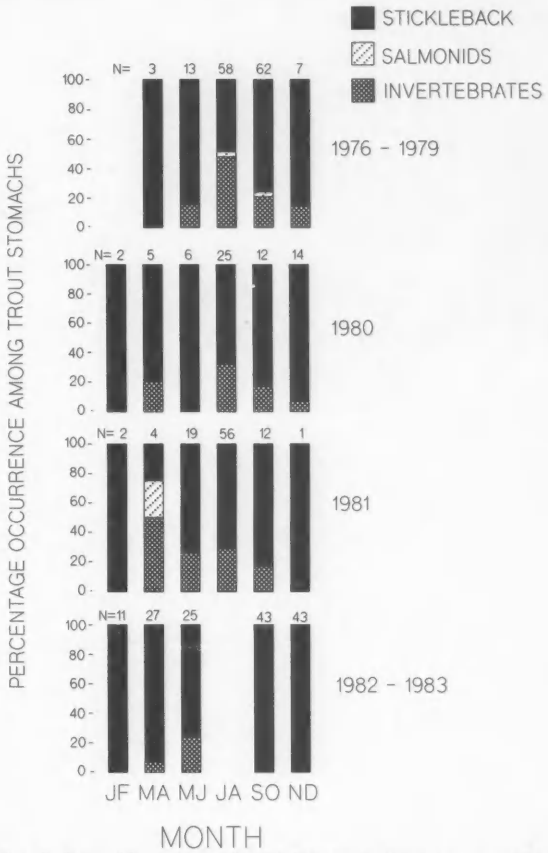

FIG. 5. Bimonthly patterns of trout diet. Vertical axes represent the proportion of trout stomachs containing the item. Proportion of fish with empty stomachs were: 1976–79 = 24%, 1980 = 23%, 1981 = 9%, 1982–83 = 17%.

Assuming an average caloric equivalence of 1.2 kcal/g tissue (Cummins and Wuycheck 1971), caloric consumption can be converted to grams:

$$[4] \quad Dg = Dcal \cdot 1.2^{-1},$$

where Dg = daily consumption in grams.

Evaluating the weight of stickleback (Ws) eaten requires several refinements to this estimate since their relative contribution to the trout diet varies both with season (Fig. 5) and with length-class of trout (Fig. 6). For each 1-cm size-class of trout (i), I derived estimates of weight of stickleback eaten per month from the following:

$$[5] \quad Ws_i = \Sigma\,[Dg_i \cdot P_i \cdot F \cdot S \cdot M],$$

where Ws_i = weight of stickleback eaten by trout of the ith size class, Dg_i = daily food consumption by trout of the ith size class (from equation [4], P_i = numbers of trout in the population of the ith length-class (from Fig. 2 and Table 3), F = frequency of trout foraging within each bimonthly interval, S = frequency of foraging trout containing stickleback in stomachs, M = number of days per month.

Comparisons between predicted and actual consumption of stickleback

To ascertain whether the calculations give a realistic estimate, I compared the estimated daily stickleback consumption by trout (from equation [5], $Ws \cdot M^{-1}$) with actual weights of stickleback in trout stomachs. Estimated daily consumption was converted to percentage body weight to compensate for differences in trout body weight; this value (ca 2.5%) is relatively constant over the range of trout lengths observed. Stomach contents were partitioned into two groups, one representing the weight of intact stickleback, and the second representing this value in addition to the reconstructed weights of partially digested fish. Complete evacuation of the stomach, which is temperature dependent, is approximately 24–48 h at 17°C and 72–144 h at 5°C (Popova 1967) and therefore one can predict that actual might approximate predicted in summer but be up to six times greater than predicted in winter. The data (Fig. 10) show that actual stickleback consumption (intact + reconstructed) is about 20% lower than the estimated in summer but up to six times higher than the estimated in autumn and winter indicating that my calculated values, which are used in the following estimates of total numbers of individuals eaten, are realistic.

Estimating numbers of stickleback consumed by trout

Number of stickleback eaten per month (Ns) for each length-class of trout can be derived from combining weight of stickleback eaten per month (equation [5] with the length frequency distribution of stickleback in the stomachs. The latter is however dependent on season and trout length (Fig. 8A, B, 9). For each bimonthly interval, I grouped trout into size-classes$_i$ (10–19 cm, 20–24 cm, 25–29 cm, 30–34 cm, 35–41 cm) and tabulated the proportion of each stickleback length group ($_j$) (7–9, 10–14, 15–19, 20–29, 30–39 mm... etc.

$$[6] \quad Ns_i = Ws_i \cdot (n_j \cdot w_j)^{-1},$$

where Ns_i = numbers of stickleback eaten per month for the ith size class of trout, Ws_i = total weight of stickleback eaten per month for ith size class of trout (from equation [5]), n_j = frequency of the jth size-class of stickleback in the stomach, w_j = the weight of the jth size-class of stickleback.

Therefore, total stickleback consumption per month (N) for the entire trout population is

$$[7] \quad N = \Sigma(Ns_i),$$

where i = 1-cm length-group of trout between 11 and 41 cm.

Weight and number of stickleback eaten

Estimated annual stickleback consumption was 145.4 kg or 308 770 stickleback. Grouping trout into size-classes shows that the intermediate-sized trout (25–29 cm) took the greatest weight of stickleback per year (Table 4). However, small-bodied trout (14–19 cm), which had the lowest yearly weight of stickleback consumption, consumed the greatest number of individuals.

There is a major seasonal component to the total stickleback consumption by trout with 64% of the weight of stickleback taken from June to August and 13% taken from November to March. Comparable values (74 and 6.8%, respectively) occur in number of stickleback eaten (Fig. 11). The peak consumption in summer results predominantly from increased temperatures and the resultant increase in predicted foraging rates.

Partitioning monthly consumption among length-groups of stickleback yields a substantive season–length interaction

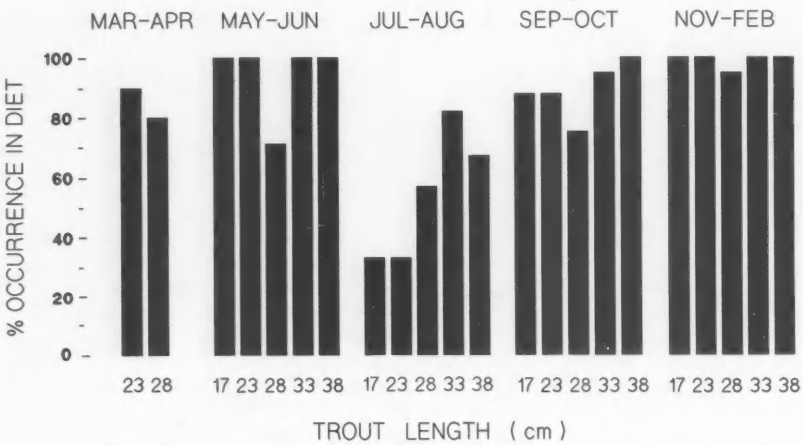

FIG. 6. Proportion of trout stomachs containing stickleback in relation to season and trout length.

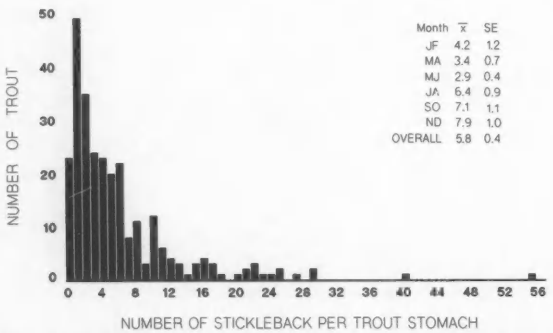

FIG. 7. Number of stickleback per trout stomach. Data from all years included.

(Fig. 12). The smallest length-class (7–20 mm) were taken predominantly in July and August, the only period when this size-group was present. Among the 20–40 mm stickleback, predation took place throughout the year with most occurring from April to October. Among larger stickleback (40–60 mm), there appears to be a contraction of the predation period to summer months. Yet in the largest length-classes (60, 70 and 80 mm), there was a progressive expansion of the predation period to encompass the entire year. The largest fish (>80 mm) are taken commonly in both winter and summer and less frequently in spring and autumn.

Total yearly predation on stickleback, partitioned into length-classes, is shown in Fig. 13A. The smallest fry (7–9 mm) are relatively infrequent in the stomachs while larger fry (10–20 mm) comprise the most common length group and account for 44.7% by number of the total predation. Among progressively larger stickleback, there is a gradual reduction in numbers consumed. Adult stickleback (>70 mm) incur the lowest predation.

Length-classes of eaten stickleback were placed in age-groups to determine age-specific mortality levels (Fig. 13B). The 0–1 year-class comprise 73% of the total predation by trout while the 3+ year-groups represent 2%. There is approximately a 75% reduction (range 67–84%) in consumption in each successive year-class.

General estimates of consumption levels relative to population size are possible. The 240 842 YOY stickleback that were eaten comprise 2.0% of the total number of individuals originally present at the post-hatching stage while total consumption (all age-classes) represent 2.6% of the original cohort. The 3112 adults eaten represent 4.1% of the total adult population in the lake.

Discussion

Previous studies of cutthroat trout indicate a variable diet including benthos, midwater zooplankton, fishes, and surface insects, the proportion of each depending on the size of the trout, the season and the occurrence of competitors (Andrusak and Northcote 1971; Armstrong 1971; Moodie 1972; Sigler et al. 1983; Skinner 1985; Luecke 1986; Loch and Miller 1988). At Drizzle Lake, stickleback are the dominant element in the diet throughout the year except among small trout during summer when insects predominate. The extent of piscivory, which is very high, has previously been observed where cutthroat trout are sympatric with Dolly Varden (Nilsson and Northcote 1981), a species also present in this lake.

Previous studies have shown that stickleback are rare in the diet of small predatory fish (<20 cm) (Frost 1954; Fortunatova 1959), presumably since spiny prey are unsuitable for young predators (Popova 1967). McPhail (1977), working with fish in Vancouver Island populations, indicated that cutthroat trout less than 20 cm rarely ate stickleback. At Drizzle Lake, these prey represented the major diet of small-bodied trout (14–19 cm) throughout most of the year. This is surprising since the stickleback from this population have exceptionally long and robust spines (Moodie and Reimchen 1976). Although juvenile coho salmon (40–60 mm SL) were abundant in the littoral region, these were uncommon in the diet (see also Moodie 1972; Loch and Miller 1988). It is probable that the paucity of suitable alternate prey in this dystrophic lake has resulted in the high consumption of stickleback by small trout and may partially explain why even smaller trout (<12 cm) are rare or absent in the lake.

The prevalence of stickleback and virtual absence of small salmonids in the cutthroat trout diet is characteristic of other piscivores in this locality. Kingfisher (*Ceryle alcyon*), common loon (*Gavia immer*), grebes (*Podiceps* spp.), and river otter

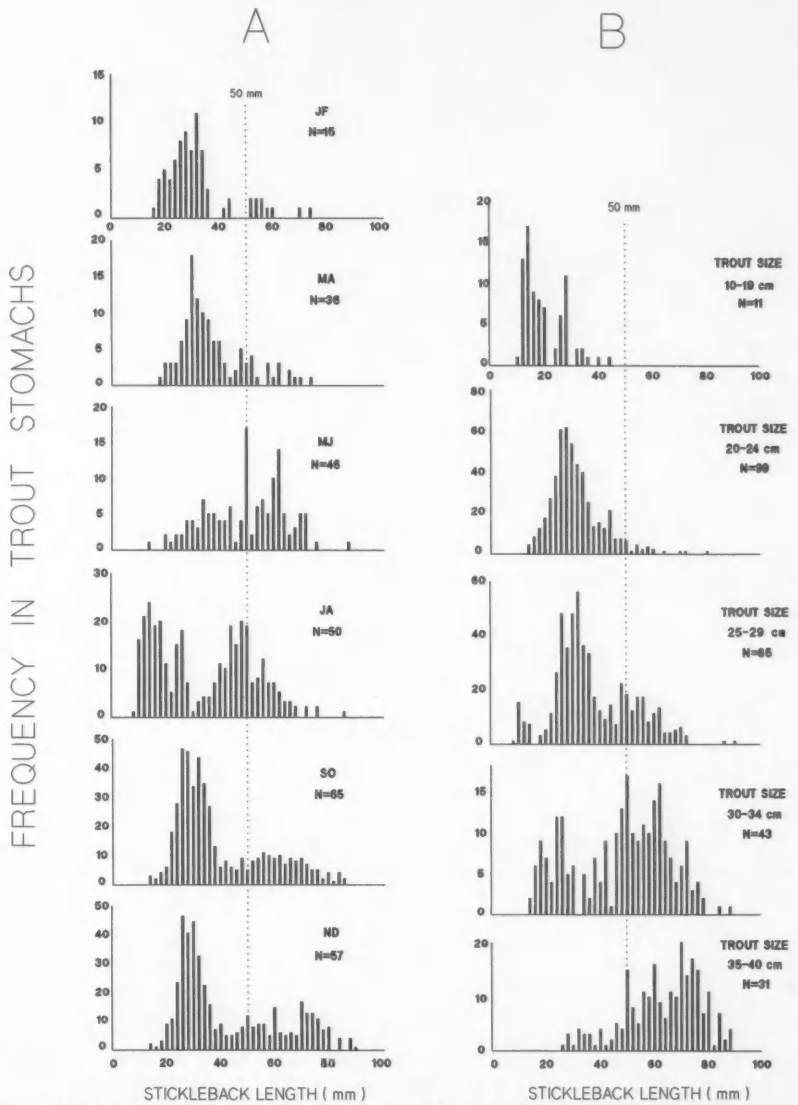

FIG. 8. Standard length of stickleback in trout stomachs at bimonthly intervals (A) and for different size-classes of trout (B). Vertical axes show numbers of stickleback in each millimetre-length-class. N = number of trout stomachs.

(*Lutra canadensis*) also forage primarily (>90% of the diet) on stickleback (Reimchen and Douglas 1984; Reimchen 1990). In northern Europe, stickleback often comprise the major element in the diet of mergansers, terns, and kingfishers (Rad 1980; Sjoberg 1989). Presumably, the small size, slow swimming speed, and abundance make stickleback a preferred prey. Such observations argue for caution in recent suggestions (O'Neill and Hyatt 1987) that number of stickleback be controlled to minimize their effect on juvenile sockeye salmon (*Oncorhynchus nerka*) with which they compete for zooplankton.

Analyses of seasonal predation levels predicted that the overwhelming majority of stickleback would be eaten during summer, primarily as a consequence of the high metabolic requirements. The extent of this major seasonal pulse would be less if, for example, trout moved offshore during summer to colder water (i.e. Andrusak and Northcote 1971) or if daily requirements were increased in winter, for example, during gonad development (Elliott and Persson 1978). Other studies of predation on stickleback (Moodie 1972; Hagen and Gilbertson 1973), have suggested that autumn, winter, and spring may have the greatest predation levels. This conclusion is based on the observation that a greater proportion of trout stomachs contained stickleback and there were greater numbers of stickleback per stomach during these cooler seasons. Both of these trends also occur in the present study. However, in winter tem-

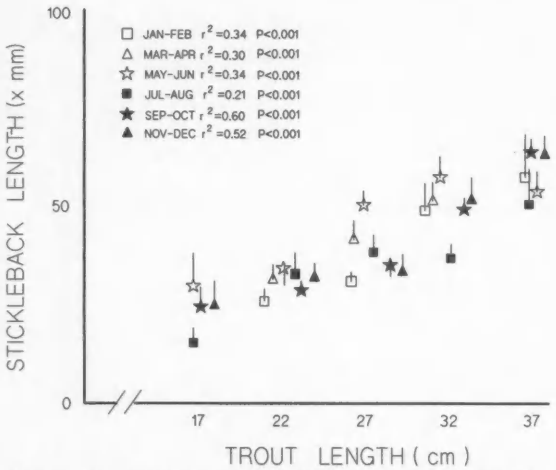

FIG. 9. Mean length (+ 95% confidence limits) of sticklebacks in stomachs in relation to trout size and bimonthly period.

peratures (e.g. near 5°C), stomachs may contain the remains of fish from 4–6 d of foraging while in summer (17–20°C), the entire contents of the stomach can be digested in less than 24 h (Molnar et al. 1967; Popova 1967). Consequently, increased occurrence of prey in autumn and winter may reflect reduced evacuation rates rather than increased predation rates.

Seasonal differences in size-specific mortality are a common feature in size-structured populations since the availability of each size-class of prey will differ over time as a consequence of body growth (Werner and Gilliam 1984 for review). Accordingly, in the Drizzle Lake population, juveniles (<20 mm) are only available during summer following the spring breeding period while large stickleback (>70 mm) are abundant throughout the year and are found in the stomachs in all seasons, particularly summer and winter. This explains part, but not all, of the seasonal variability in consumption levels. Predation on the 40–60-mm size-class appeared to be restricted to summer even though this size-class can be found year-round. One characteristic of this class is that it is primarily limnetic (T. E. Reimchen, pers. observ.). Increased predation would occur if trout, which are primarily littoral foragers, moved offshore during summer. Such movement has been observed in several localities, presumably as a response to rising littoral temperatures (Andrusak and Northcote 1971). Sharp reduction in littoral fyke net captures during July are consistent with this suggestion (Fig. 4). Results from gill nets show that most trout activity in autumn and winter is within 100 m of shore while in spring and summer, it is equally distributed in shoreline and open water regions, consistent with increased limnetic predation levels during these periods.

Total yearly consumption estimates are subject to various errors. For example, organisms differ substantially in their caloric value, from 0.9–1.6 kcal·g^{-1} (Cummins and Wuycheck 1971). I assumed an average food value of 1.2 kcal·g^{-1} since this comprised a mixture of both fish and insects. Increasing this value to 1.35 kcal. g^{-1}, that is, if all the prey were similar in nutrient value to an average fish, would reduce total stickleback consumption by 11%. Any movement of the trout into cooler waters during summer (when 65% of the total yearly predation is predicted), would result in a reduction in metabolic rate and a corresponding reduction in consumption. Lowering average temperature by 3°C throughout the year reduces predicted consumption by 50%. I also computed a range of predation estimates by arbitrarily changing length of trout. A 5-cm reduction in trout length decreases yearly weight consumption of stickleback by 40% but increases numbers by 80%. A 5-cm increase in length (with the resultant loss of the 14–19-cm class) would increase yearly weight of stickleback eaten by 60% but reduce numbers of stickleback eaten by 22% (since fewer small trout are present to eat small stickleback). Small changes in predator size therefore alters the size-specific mortality and potential selective pressures on the prey.

The age-specific predation curve showed a 75% average reduction in consumption of each successive year-class of stickleback. This curve is not substantially different from the survivorship curve assumed to occur in many fish populations. Such a relationship could imply that trout are consuming the same proportion of each age-class in the stickleback population. Many predatory fish are opportunistic and the proportion of taxa in the stomach commonly tracks the abundance of prey (Frost 1954; Popova 1967). However, predatory fish are gape-limited (Popova 1967; Werner 1974; Zaret 1980; Tonn and Paszkowski 1986; Werner and Hall 1988) and therefore, the prey-size consumption curve in size-structured populations will be dependent on the size distribution of the predator (e.g. Nielsen 1980). The reduction in predation levels on the larger size-classes of stickleback probably results from a corresponding reduction in progressively larger trout capable of swallowing the larger stickleback. A 70-mm stickleback with spines erect has a cross-sectional diameter greater than the gape of most trout in the lake and is usually rejected by the trout either during pursuit or during manipulation (T. E. Reimchen, unpubl. data).

Stickleback fry (7–9 mm) are the most abundant length-class in littoral regions during summer yet they represent only 2.6% of the diet. Fry will be digested quickly and would be less detectable in the stomachs but I suspect that this is not a significant factor. While these fry are larger than the minimum

TABLE 3. Values used for calculating total stickleback consumption by cutthroat trout.

	J	F	M	A	M	J	J	A	S	O	N	D
Population estimates of trout (*N*)	115	115	115	110	200	250	250	200	100	90	90	115
Frequency of trout foraging (%)	46	46	47	77	100	100	100	100	100	100	100	100
Temperature °C	4.9	4.9	5.5	7.2	10.5	14.5	16.9	16.9	14.4	10.0	4.5	4.0
Frequency of stickleback in diet (%)												
Trout size = 14–19 cm	100	100	85	85	75	75	75	75	85	85	100	100
20–24 cm	100	100	85	85	86	86	71	71	81	81	100	100
25–29 cm	100	100	95	95	75	75	72	72	84	84	88	88
30–34 cm	100	100	95	95	88	88	74	74	91	91	100	100
35–41 cm	100	100	100	100	100	100	100	100	100	100	100	100

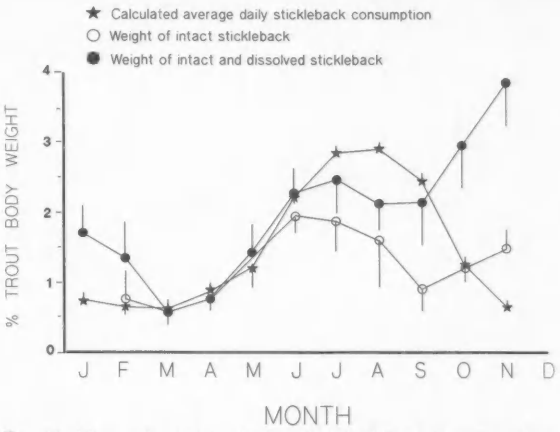

FIG. 10. Mean values (± 1 SE) of predicted stickleback consumption in comparison to actual weight of stickleback in trout stomachs. All weights of stickleback converted to percent trout body weight.

TABLE 4. Yearly consumption of stickleback by cutthroat trout at Drizzle Lake.

	Total consumption of stickleback	
Trout size (cm)	Weight (g)	Numbers
14 – 19	10 558	149 504
20 – 24	36 506	72 465
25 – 29	69 984	63 159
30 – 34	21 670	17 723
35 – 41	15 673	5 919
Total	145 391	308 770

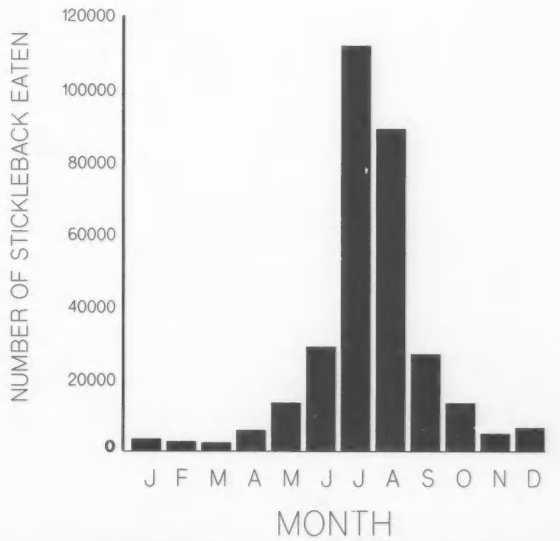

FIG. 11. Monthly consumption of stickleback by trout at Drizzle Lake. Histogram shows average consumption using representative parameters (see Table 3).

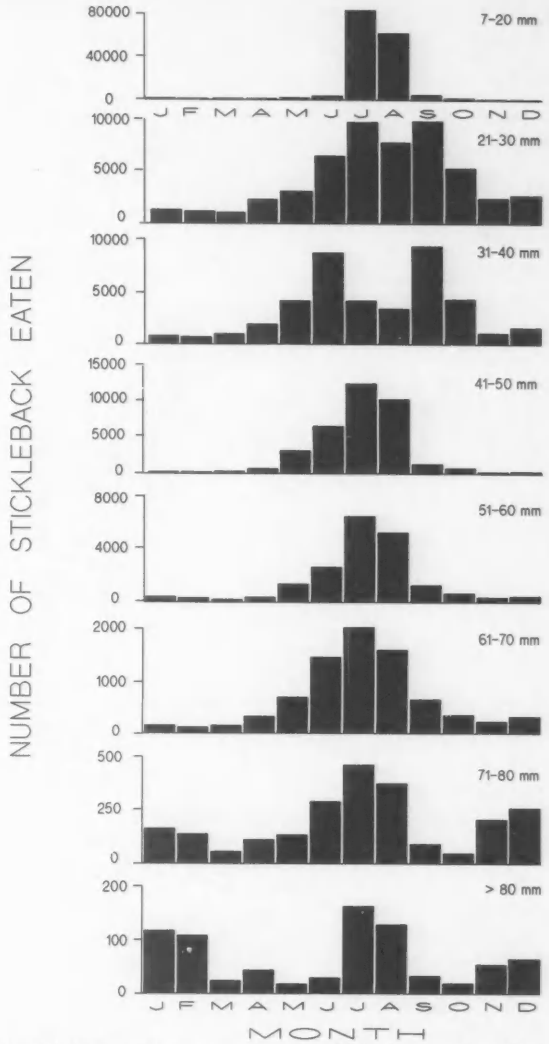

FIG. 12. Size-specific consumption of stickleback at monthly intervals. Vertical axes show absolute number of stickleback eaten. Note differences in scale.

prey size (2–3 mm) (Skinner 1985), they are smaller than the average stickleback (17 mm) observed in small trout during July and August when fry were most abundant. Popova (1978) found that prey of piscivorous fish were generally from 10–40% the body length of the predator. Stickleback fry (9 mm) would be about 5% the body length of a small-bodied trout (17 cm) and therefore potentially outside the preferred prey-size distribution. In addition, fry may be unavailable to these foragers if microhabitats provide refuge (for example, Foster et al. 1988).

Yearly prey consumption by predators relative to total prey population are highly variable within and between species (see Vetter 1988 for review). Lake whitefish (*Coregonus clupeaformis*) had an average natural mortality rate of 49% per year-class (range 18–86%) although it is unknown what proportion of this was from predators (Healey 1975). In Finland, northern pike (*Esox lucius*) consumed about 40% of the older age-classes

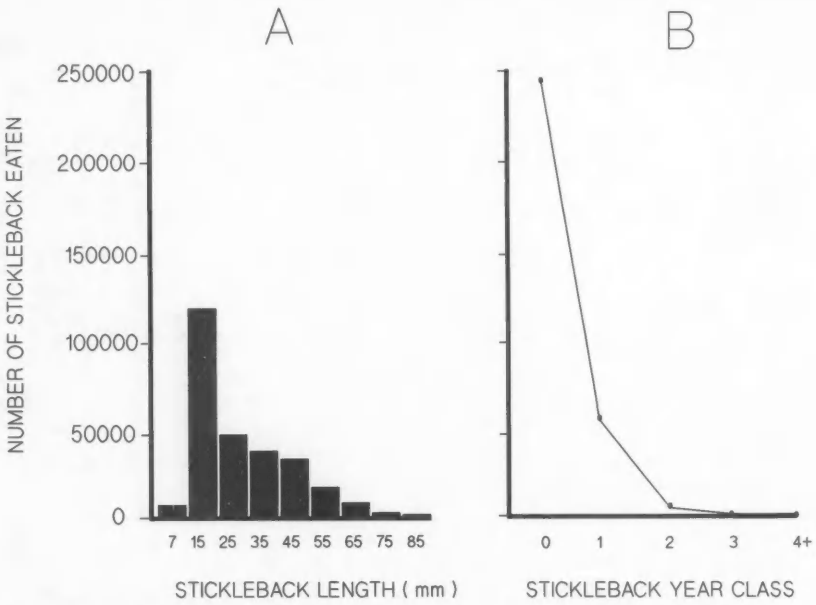

FIG. 13. (A) yearly size-specific (B) age-specific consumption of stickleback at Drizzle Lake from trout predation.

(≥2 yr) of perch (*Perca fluviatilis*) (Rask and Arvola 1985). In the Volga delta, four species of predaceous fish collectively consume about 11% of the 0+ year-class and 1.5% of the 1+ and 2+ year-classes of a population of roach (*Rutilus rutilus*) (Popova 1978). In Paul Lake, British Columbia, rainbow trout (*Oncorhynchus mykiss*) (formerly *Salmo gairdneri*) consume less than 7.5% (possibly as low as 0.15%) of population of redside shiners (*Richardsonius balteatus*) (Crossman 1959). My estimate for trout consumption of the adult stickleback population (4%) is therefore low. This may be the consequence of the relatively large body size of the stickleback. Yet, predation on the sub-adult and the juvenile population was only 2% of the initial maximum potential recruitment. This value could be a substantial underestimate since an unknown proportion of the initial egg and fry recruitment are taken by conspecifics, odonate naiads, and Dolly Varden (Reimchen 1990).

In an earlier study at Drizzle Lake, total fish consumption by 14 species of avian piscivores was estimated at 295–573 $kg \cdot y^{-1}$ (Reimchen and Douglas 1984). Therefore, trout are consuming about 40% of that taken by birds. In a large oligotrophic lake in southern Sweden, predatory fish consumed about twice the weight of fish taken by 12 species of resident avian piscivores (Nilsson and Nilsson 1976). Presumably the proportion taken by each group of predators will differ among localities according to the suitability of the prey, the relative abundance of the piscivores and the characteristics of the habitat. Drizzle Lake is deeply colored with maximum underwater visibility of about 1 m (T. E. Reimchen, pers. observ.) which could favour different foraging techniques to those in clear water habitats.

In summary, the evaluation of stickleback–trout associations in this locality demonstrates that mortality differs with season, with lake position, with predator body size, with prey body size, and with the interactions among these factors. This could be expected in size-structured populations. These data establish a demographic framework from which evolutionary forces can be evaluated and clearly indicate the potential complexity of interactions in this relatively simple aquatic community.

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Thermal Structure of a Multibasin Lake: Influence of Morphometry, Interbasin Exchange, and Groundwater

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Robertson, D. M., and R. A. Ragotzkie. 1990. Thermal structure of multibasin lake: influence of morphometry, interbasin exchange, and groundwater. Can. J. Fish. Aquat. Sci. 47: 1206–1212.

Interbasin differences which develop below the upper mixed layer in small multibasin lakes provide the unique opportunity to examine how a lake's morphometry affects the rates of certain processes and the distribution of certain physical, chemical, and biological parameters without the confounding inter-lake dissimilarities encountered when examining different lakes. To determine if the variability in basin morphometry can be the primary factor causing interbasin differences, we examined the thermal structure of Trout Lake, Wisconsin, during summer and winter stratification and quantitatively explained the observed differences in temperature among basins. Differences in hypolimnetic temperature during summer stratification and differences in the temperature of the entire water column in the winter were primarily due to the morphometric differences among basins. During winter, variations in the temperature among basins were also influenced by interbasin exchange and differences in groundwater inputs. Therefore, this multibasin lake can provide the opportunity to test for morphometric affects without the confounding inter-lake dissimilarities encountered when examining different lakes.

Les différences entre bassins qui apparaissent sous la couche mixte supérieure dans les lacs à petits bassins multiples offrent une occasion unique d'examiner l'influence de la morphométrie d'un lac sur le déroulement de certains processus et la distribution de certains paramètres physiques, chimiques et biologiques, abstraction faite des différences marquées qui ressortent lors d'études de lacs différents. Afin de déterminer si la variabilité de la morphométrie d'un bassin peut être le principal facteur à l'origine des différences entre bassins, nous avons examiné la structure thermique du lac Trout, Wisconsin, pendant la stratification estivale et hivernale et expliqué de façon quantitative les différences de température observées entre les bassins. Les différences de température de l'hypolimnion pendant la stratification estivale et de l'ensemble de la colonne d'eau en hiver étaient principalement attribuables à des différences morphométriques entre les bassins. Pendant l'hiver, les variations de température entre les bassins étaient également influencées par les échanges entre les bassins et les différences d'apport d'eaux souterraines. C'est pourquoi ce lac à bassins multiples nous donne l'occasion de vérifier l'influence de la morphométrie d'un lac, abstraction faits de différences déconcertantes que l'on rencontre lors de l'étude et de la comparaison de différents lacs.

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Lake morphometry exerts a major control over a range of lake features and processes, such as exhibited in areal hypolimnetic oxygen depletion models (Cornett and Rigler 1980) and morphoedaphic indices (Ryder 1965). When comparing among lakes with different morphometries, however, other factors such as climate, water color and clarity, sediment chemistry, and biological interactions can confound the assignment of differences due to morphometry alone. Multibasin lakes provide an opportunity to examine the effects of morphometry while minimizing other confounding inter-lake factors. To test this idea, we examined the thermal structure of the basins in Trout Lake, Wisconsin and quantitatively determined the heat budget for each basin and hypolimnion to determine whether the differences in morphometry alone could explain the differences in water temperatures. We examined the thermal features because they appear to be the most directly linked to morphometry, and heat being an easily measured parameter could be quantitatively evaluated. In analyzing the differences among basins, we first determined the extent to which the differences in the thermal structure could be attributed to differences in morphometry alone. Then other factors, including interbasin exchange and basin specific parameters such as light extinction and groundwater inputs, were examined.

Large multibasin lakes, such as Lake Erie and Lake Champlain, often exhibit interbasin differences in epilimnetic and hypolimnetic temperatures, dissolved oxygen, and other chemical properties (Potash et al. 1969; Boyce et al. 1980; Charlton 1980; Schertzer 1987). Few studies have dealt with the interbasin variability in small lakes (Boyce et al. 1980; Stauffer and Armstrong 1984). The extent of interaction between adjacent basins depends on the sill depths, the density stratification near the sill, and the time allowed to horizontally mix water between basins (Schertzer 1987). Small multibasin lakes should have relatively uniform epilimnetic conditions due to the short mixing time required to completely mix epilimnetic water, and because no physical barrier prevents lateral mixing between basins. Below the sill depth, water in a stratified lake is physically restricted from moving horizontally between basins. However, if no density stratification exists, the entire water col-

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umn should remain in free circulation and the exchange of deeper water would be relatively unrestricted.

During stratified conditions, isolation of water in separate basins may result in physical, chemical, and biological differences among basins depending on basin specific parameters including morphometry, light penetration, and possibly groundwater input in each basin (Boyce et al. 1980; Stauffer and Armstrong 1984). In summer, internal oscillations of the thermocline can occasionally provide the vertical movement necessary for interbasin exchange below the sill depth (Boyce et al. 1980; Bartish 1987). Interbasin exchange tends to reduce the differences among basins.

Study Area

Trout is a dimictic, oligotrophic lake located in Vilas County, Wisconsin, 46°20′N, 89°41′W (Fig. 1 and Table 1). It is divided into two parts, north and south Trout Lake, connected by a narrow passage. North Trout Lake contains a single basin (N Basin); whereas, South Trout Lake contains three basins (SE, SW, and SN Basins). The basins are separated by sills that range in maximum depth from 6.5 to 8.5 m which extend above the summer thermocline depth.

Methods

Water temperature measurements and secchi depths were collected intermittently at the deepest location in each basin from July 1981 to August 1986. In addition, more extensive weekly measurements were made in each basin from May to September 1982 during which light extinction measurements were also collected. Water temperatures were measured at 1–m intervals with a MontederoWhitney TC-5C underwater thermistor thermometer. The thermometer had a precision of ±0.05°C, and was calibrated bimonthly with a certified mercury thermometer. Transmitted visible light was measured at 1-m intervals with a LICOR underwater light meter, Model 185-B.

Temperature transects were made along Transect A (Fig. 1) approximately monthly from May 1982 to April 1983. Water separation between basins can often be observed from isotherm discontinuities from temperature transects taken across sills. Horizontal temperature gradients can result from wind stress in summer or differential heating from the sediments and groundwater intrusion in winter. These gradients are a measure of both vertical and horizontal motions that can lead to interbasin exchange (Stewart 1972; Bartish 1987).

Thermocline oscillations were monitored using Ryan Model J recording thermometers placed in the metalimnia in the SE and SW Basins (Fig. 1). These thermometers had a precision of ±0.5°C.

For purposes of comparison and calculation, we define the thermocline depth as the depth where the maximum rate of decrease in temperature occurs and the upper bound of the metalimnion as the depth at which the temperature changes are at least 1°C per metre. The depth of the maximum rate of decrease was determined from the first and second derivatives of a cubic spline equation fit to average temperature profiles.

Average vertical heat fluxes per unit area through each metre in the water column were calculated similar to Jassby and Powell (1975). This method involves first calculating heat contents per unit area below each depth for each sampling period. For each depth, calculated heat contents are then regressed against time in days. The slope of the regression represents the average heat flux per day through a given depth over the entire time period. An average heat flux was used to remove the effects of an oscillating thermocline.

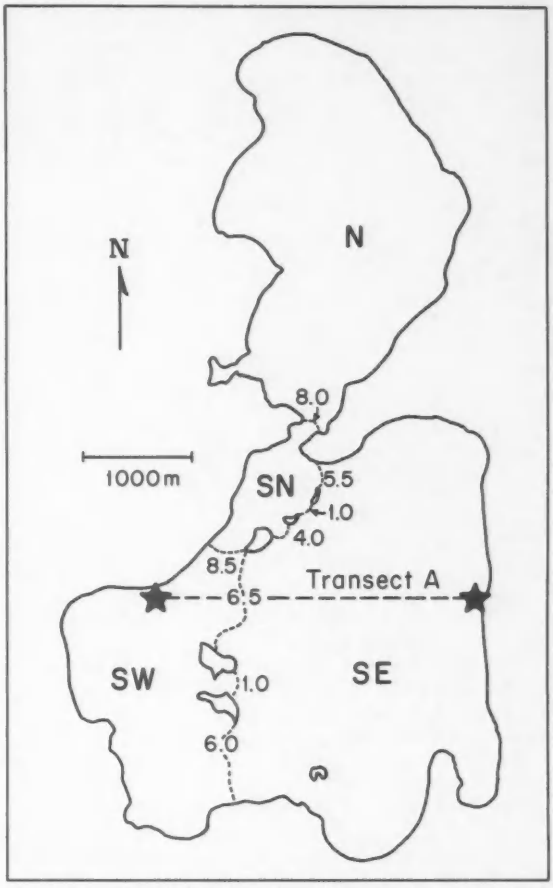

FIG. 1. Trout Lake indicating basins, sills separating basins (dotted lines), Transect A (dashed line), and location of recording thermometers (stars). Each sill is labelled with its maximum depth.

Results and Discussion

The extent of water separation and thermal variations among basins depends on the degree of stratification near the sill depths. During spring and fall, nearly isothermal conditions occurred throughout the lake. Given the absence of stratification, wind induced mixing, and similar temperature profiles among basins, free exchange of water between basins seems evident. Therefore, throughout the remainder of this section, we will primarily discuss the thermal structure of the lake during (1) the summer stratification period of 1982 and (2) the winter ice covered period of 1983. However, we will compare the results for these periods with those of other years with sufficient data and the average based on all years whenever possible.

Summer

Stratification

Early summer stratification developed as a broad metalimnion extending from approximately 3 to 10 m throughout the

TABLE 1. Morphometries of the separate basins in Trout Lake and aggregated morphometries for all South Trout Lake and the entire lake. The mean depth below a specified location represents the relative volume below that depth.

Basin	Maximum depth (m)	Surface area (m^2*10^5)	Total volume (m^3*10^6)	Basin length (km)	Mean depth (total) (m)	Mean depth (below 6.5 m)	Mean depth (below 11.5 m)
SE	35.7	77.0	132.5	4.01	17.2	14.7	12.6
SN	22.6	5.7	8.1	1.31	14.2	9.6	6.9
N	27.5	51.7	71.6	3.57	13.9	11.5	9.0
SW	19.0	26.8	22.2	2.73	8.3	5.5	3.6
Aggregations							
South Trout	35.7	109.1	163.1				
Total	35.7	160.8	235.1				

lake (Fig. 2a). This layer descended and the temperature gradient increased in a step-like response to episodic wind mixing events. After the thermocline descended past the sill depths (6.5 to 8.5 m), consistent differences in the thermal structures were found among the metalimnia and hypolimnia of the basins (Figs. 2b and 3a).

Throughout summer the top of the metalimnion in the SE Basin was consistently deeper than the tops of the metalimnia in the other basins. On average the top of the metalimnion in the SE Basin during 1982 was between 8 and 9 m, whereas those for the other basins were between 7 and 8 m (Fig. 3a and Table 2). The deeper metalimnion in the SE Basin was evident in almost all other years studied which resulted in a similar pattern exhibited in the average summer profiles for 1981 to 1986 (Fig. 3c and Table 2).

The temperature gradient within the metalimnia also varied consistently. The gradient was larger for the SE and N Basins than for the SW and SN Basins (Fig. 3a). This difference was present in most years, especially in the summer of 1986 (Fig. 3b), and is evident in the average profiles from 1981 to 1986 (Fig. 3c). The actual thermocline depths in the metalimnia were difficult to define because of the differences in the temperature gradients; however, the thermocline of the SE Basin was usually deeper than those of the other basins (Table 2). In 1982, the average summer thermocline depth of the SE Basin was only slightly deeper than those of the other basins, but in general, its summer thermocline depth was about 1 m deeper than thermocline depths of the other basins (Table 2). In 1986, the calculated thermocline depths in the SW and SN Basins were deeper than thermocline depths computed for the larger basins (Table 2). The thermocline depths of the average profiles for 1986 actually appear very similar (Fig. 3b). This discrepancy demonstrates the difficulty in estimating thermocline depths for profiles with different temperature gradients.

The most apparent difference in thermal structure was that the hypolimnion of the SW Basin became 3°C warmer than the hypolimnia in the other basins (Fig. 3a). The temperatures of the hypolimnia varied among years; however, the SW Basin consistently became almost 3°C warmer than the other basins (Fig. 3c and Table 2).

During summer stratification when wind forced oscillations were absent, horizontally uniform temperatures occurred throughout the epilimnion (for example, see Fig. 2b). Average secchi depths ranged from 4.7 m in the SW Basin to 5.5 m in the N Basin (Table 3). The similarity found in secchi depths was also observed for transmitted light. The average percent of incident light at 11 m ranged from 1.9% in the SW Basin to 2.9% in the N Basin (Table 3). Light extinction, however, varied considerably in the hypolimnia.

Metalimnetic differences

Maximum thermocline depths of temperate lakes are primarily a function of the lake's "unobstructed fetch" (Ragotzkie 1978; Straskraba 1980; Shuter et al. 1983). The unobstructed fetch is the linear distance over water that wind can travel unaffected by the shoreline, islands, or shallow shoals or sills. The unobstructed fetch of an individual basin in a multibasin lake is difficult to define because wind often travels over two basins. Waves and vertical mixing often are affected by constrictions and islands between basins and by underwater sills. Thus, the effective fetch of an individual basin can be longer than the length of that basin, but shorter than the total distance from shore to shore. Several empirical relationships between thermocline depth and fetch have been developed, for example equations (1) and (2). Z is the thermocline depth in metres and F is the maximum fetch in kilometres.

$$(1)\quad \mathrm{Ln}\, Z = 0.298 \cdot \mathrm{Ln}\, F + 1.82 \qquad \text{(Shuter et al. 1983)}$$

$$(2)\quad Z = 4.0 \cdot (F)^{0.5} \qquad \text{(Ragotzkie 1978)}$$

Using the basin length as the maximum fetch, equations (1) and (2) predict the thermocline depths of the basins to differ, especially in the much smaller SN Basin. However, the similarity in thermocline depths and the upper depth of the metalimnia suggest other factors are important, such as interbasin exchange resulting in the effective fetch of the smaller basins being much longer than their actual length. Interbasin exchange should continuously occur down to the maximum depth of the sill separating adjacent basins. In addition, limited exchange below the sill depths should occur with wind driven currents associated with internal seiches. The deepest sill separating the SN Basin from the N and SE Basins, is 8 m between the SN and N Basins. Therefore, because the N Basin has a thermocline depth deeper than 8 m and because a thermocline shallower than 8 m in the SN Basin could not be independently maintained, i.e. gravitational forces would cause the colder and denser water of the metalimnion of the smaller SN Basin to flow horizontally into the N Basin, the shallowest thermocline depth expected for the SN and SW Basins is just below the maximum sill depth. In addition, internal seiching should cause the thermocline depth to be slightly deeper than that.

Evidence for interbasin exchange associated with internal seiches comes from recording thermometers placed at the ther-

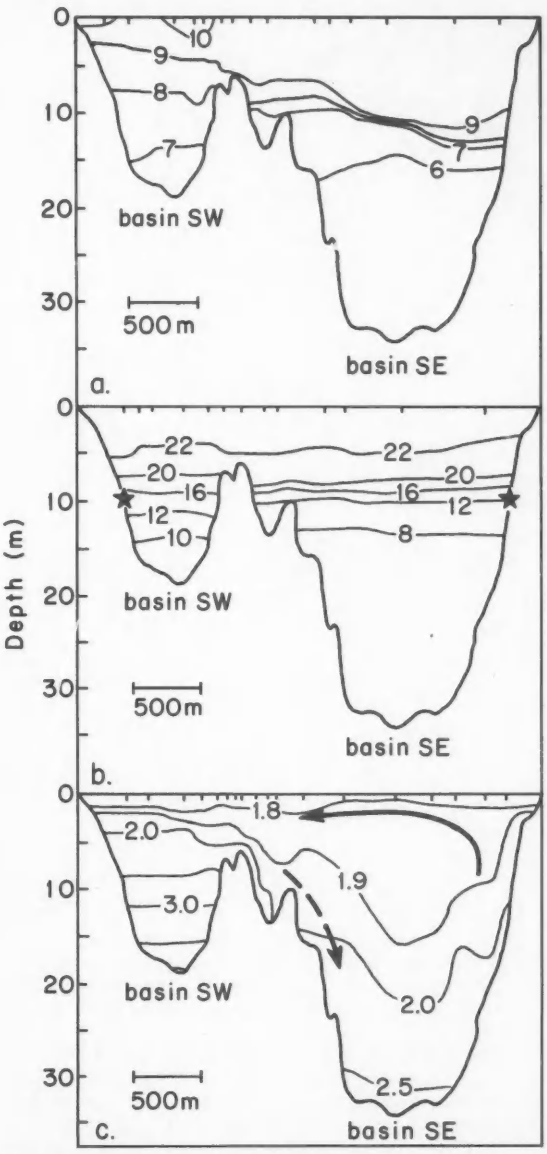

FIG. 2. Temperature Transect A across the SW and SE Basins. Isotherms are labelled in °C. Sounding sites are indicated on the surface by ticks. (a) Early summer transect, demonstrating tilted thermocline. (b) Summer transect from 5 August 1982. Stars indicate location of recording thermometers. (c) Winter transect from 25 March 1983. Arrows in the figure represent the proposed water circulation pattern in the winter.

mocline depth at opposite ends of the lake (Transect A, Fig. 1). These temperature records which were almost consistently 180° out of phase (Fig. 4) suggest the thermocline oscillated in a uninodal manner in the SE and SW Basins. In addition, Fourier spectral analysis demonstrated a significant period at 12.7 h in each basin, which is very near to the calculated period for a uninodal seiche in all of South Trout Lake of 11.7 h. The maximum vertical amplitudes of the oscillations at the shorelines were calculated to be about 2.7 m and were usually much less (Fig. 4). Because the sill between the SE and SW Basins is located closer to the node of the seiche, the vertical motion at the sill was estimated to be less than 2.5 m below the sill and generally less than 1.5 m. Thus, during the period of maximum stratification, interbasin exchange between the SE and SW Basins can occur as much as 2.5 m below the sill depth, i.e. down to about 9 m. Although no measurements of internal seiches were made between the SN and N or the SN and SW Basins, these basins appear effectively separated about 2 m below sill depth, i.e. at a depth around 10 and 10.5 m, respectively. Therefore, we expect and in fact observe the thermocline depths of the smaller SN and SW Basins to be between the depths of the larger adjacent basins, N and SE Basins, and a depth slightly deeper than the separating sills (Table 2). The fetch of the N Basin should effectively control the thermocline depths of the N, SN, and SW Basins because the sills separating the N, SN, and SW Basins are deeper than the sills separating the SE Basin from the other basins. As expected the thermocline depths of the SN and SW Basins were similar to that of the N Basin (Table 2). The upper depths of the metalimnia of the SN and SW Basins were also very similar to the upper depths of the metalimnion of the larger N Basin (Table 2).

Interbasin exchange associated with internal seiches would also result in weaker temperature gradients in the smaller SW and SN Basins. If interbasin mixing is incomplete the resulting thermal structure in the smaller basins will be between that based on their own fetch and that of the larger adjacent basin. Therefore, the net result of limited mixing for the smaller basins will be a broadened metalimnion with a more gradual change in temperature which was observed.

Differences in hypolimnetic temperature

At the onset of summer stratification, the hypolimnion of the SW Basin was less than 1°C warmer than other basins. However, as summer progressed, the basin became more than 3°C warmer than the others (Fig. 3a). This difference among basins varied slightly among years (Table 2). In other years, smaller differences also occurred among the other basins, demonstrated by the differences at 17 m (Table 2). Temperature differences below the sill depths may develop due to morphometric differences among basins alone, if the heat flux per unit area into each hypolimnion is similar, or due to morphometric differences plus external factors influencing each basin uniquely. If the heat flux per unit area into each hypolimnion was similar then any resulting difference in temperature can be explained by the same amount of heat being distributed into different volumes. In addition, external factors, such as solar radiation penetrating into the hypolimnia and differential groundwater input, may influence each basin differently. In contrast, interbasin exchange tends to reduce differences among basins.

Morphometric effects — To determine whether the differences in basin morphometry alone could explain the differences in hypolimnetic temperatures, we calculated the average vertical heat flux per unit area into the hypolimnion of each basin. An average summer heat flux was determined for the period from mid-June, when the thermocline descended below the sill depths, to the end of July, when the entire wter column reached its maximum heat content. We originally defined the top of the hypolimnion at 11.5 m in each basin because the 1°C criterion for the bottom of the metalimnia often was rather vague and extended over several metres (Fig. 3). Interbasin transfer was

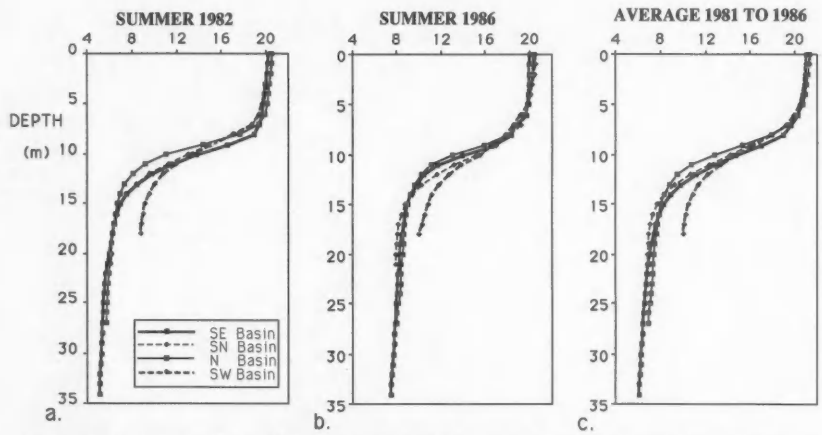

FIG. 3. Average temperature profiles from each basin for maximum summer stratification. (a) Summer 1982, calculated from an average of nine profiles from each basin from 16 June to 18 August. (b) Summer 1986, calculated from an average of seven profiles from each basin from 9 June to 20 August. (c) Average summer stratification from 1981 to 1986.

TABLE 2. Comparison of summer thermal structure: upper depth of the metalimnion, thermocline depth, and hypolimnion temperature. The upper depth of the metalimnion is the shallowest depth interval with more than a 1°C per metre change in temperature. The hypolimnion temperature is represented by the temperature at 17 m.

Year	1982	1981	1983	1984	1985	1986	Average
Upper depth of metalimnion (m)							
SE	8.5	7.5	7.5	8.5	10.5	8.5	8.5
SN	7.5	7.5	6.5	8.5	7.5	7.5	7.5
N	7.5	7.0*	6.5	7.5	7.5	8.5	7.4
SW	7.5	7.5	6.5	8.5	7.5	8.5	7.6
Thermocline depth (m)							
SE	9.1	9.2	9.2	11.6	12.6	9.6	10.2
SN	8.5	8.5	7.7	8.6	10.8	10.4	9.1
N	9.0	7.8	8.6	10.5	10.5	9.3	9.3
SW	8.4	8.5	8.3	8.8	11.8	10.5	9.4
Hypolimnion temperature at 17 m							
SE	6.5	7.2	7.3	8.3	7.8	8.7	7.6
SN	6.4	7.3	6.3	7.5	6.9	8.2	7.1
N	6.4	7.5	7.6	8.4	7.7	8.7	7.7
SW	8.9	9.6	10.0	11.4	10.5	10.3	10.1

*1°C Criteria extended over a wider range.

TABLE 3. Comparison of water transparency, based on average secchi depth and percent of incident light at depth. Each average value was computed from seven measurements or profiles from mid June until the end of July in 1982.

Basin	SE	SN	N	SW
Secchi depth (m)	4.9	5.1	5.5	4.7
Percent of incident light at 11 m	2.5	2.0	2.9	1.9

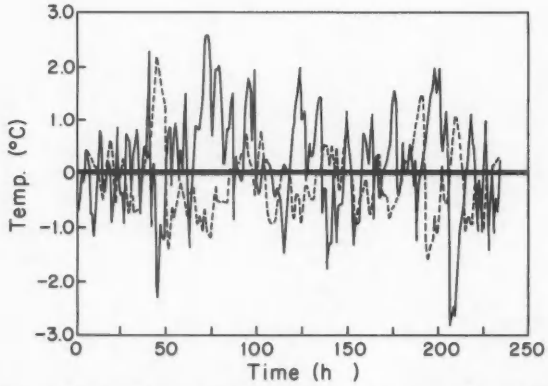

FIG. 4. Resultant thermograph from recording thermometers in the SE Basin (solid line) and the SW Basin (dashed line) from early July with heating trend removed. Curves were made using hourly measurements.

previously shown not to occur down to this depth. In order to account for the widely differing areas at the top of the hypolimnia, the mean depth below a specified location is used to describe the average volume per unit area below that depth (Table 1). Therefore, the hypolimnion of the SW Basin with a mean depth of 3.6 m has the smallest relative volume below 11.5 m and the hypolimnion of the SE Basin with a mean depth of 12.6 m has the largest relative volume. During 1982, the average daily heat fluxes through 11.5 m in the SN, N, and SW Basins were 17.8, 17.9, and 13.8 cal·cm^{-2}·d^{-1}, respectively, while that for the SE Basin was 24.2 cal·$^{-2}$·d^{-1}, a value significantly different from that of the first three basins using an analysis of covariance test for equality of slopes ($p \leq 0.05$). Similar computations for other years demonstrated similar patterns (Table 4). For the five years from 1982 to 1986, the average daily heat flux through 11.5 m in the SE, SN, N, and SW Basins was 23.5, 14.5, 14.5, and 14.1 cal·cm^{-2}·d^{-1}, respectively (Table 4). Therefore, in view of the similarity in vertical heat fluxes in the SN, N, and SW Basins, it seems clear that the primary factor causing the observed variation in temperatures

TABLE 4. Average summer heat flux ($cal \cdot cm^{-2} \cdot d^{-1}$) comparison through 10.5, 11.5, and 12.5 m in each basin. Seasonal values are computed from profiles taken between mid June and the end of July.

		Basin			
Year	Depth	SE	SN	N	SW
1982	10.5	32.5	25.7	22.7	16.8
	11.5	24.2	17.8	17.9	13.8
	12.5	19.4	12.9	14.7	12.1
1983	10.5	36.0	26.7	25.8	22.0
	11.5	29.5	19.1	22.5	18.3
	12.5	25.5	13.3	20.3	16.1
1984	10.5	24 9	21.1	15.7	16.9
	11.5	20.6	15.3	11.7	13.1
	12.5	17.6	11.9	9.1	10.7
1985	10.5	34.7	17.2	13.9	19.4
	11.5	27.3	10.4	10.3	15.7
	12.5	21.1	6.8	7.9	13.2
1986	10.5	19.9	15.8	12.7	12.9
	11.5	15.7	10.0	10.0	9.8
	12.5	12.6	6.4	8.9	7.9
Average	10.5	29.6	21.3	18.2	17.6
	11.5	23.5	14.5	14.5	14.1
	12.5	19.2	10.3	12.2	12.0

among these three basins is a similar amount of heat being distributed into three hypolimnia with differing relative volumes or mean depths. A similar conclusion is found using the top of the hypolimnia defined as 10.5 or 12.5 m (Table 4).

The much larger heat flux through 11.5 m in the SE Basin suggested other factors were operating in this basin. As the largest basin with the longest fetch and the deepest thermocline, the SE basin is undoubtedly subject to a higher total wind energy and more vigorous vertical mixing. As the top of the metalimnion of the SE Basin was approximately 1 m deeper than in the other basins, the heat flux from the epilimnion would occur about 1 m deeper in that basin. To correct for this descrepancy, we compared the heat flux through 11.5 m in the SN, N, and SW Basins with that at 12.5 m in the SE Basin. The heat flux through comparable depths in 1982 would then be 19.4, 17.8, 17.9, and 13.8 $cal \cdot cm^{-2} \cdot d^{-1}$ in the SE, SN, N, and SW Basins, respectively. A similar comparison for all five years gave the average daily heat fluxes in the SE, SN, N, and SW Basins to be 19.2, 14.5, 14.5, and 14.1 $cal \cdot cm^{-2} \cdot d^{-1}$, respectively. Therefore, correcting for the difference in thermocline depth explains much of the larger heat flux into the SE Basin.

External effects – Solar radiation and groundwater contributions are expected to have small effects on the differences that develop among the basins in the summer. The penetration of solar radiation was very similar among basins with only about 2% of the incoming radiation reaching 11 m (Table 2). The degree to which groundwater can alter a lake's thermal structure depends on the difference between lake and groundwater temperatures and the quantity entering the lake (Reynolds 1975). The temperature of groundwater deeper than 8 m below the water table (the water table coincides roughly with the lake surface) near Trout Lake is between 6 and 7°C throughout the year (Kenoyer 1986). Therefore, because groundwater temperatures were very similar to the hypolimnetic temperatures, the influence of groundwater in the summer is expected to be minimal.

In summer, epilimnetic conditions were horizontally uniform and the differences that developed in the thermal structures of the hypolimnia primarily resulted from differences in basin morphometry, after correcting for differences in upper depth of the metalimnia.

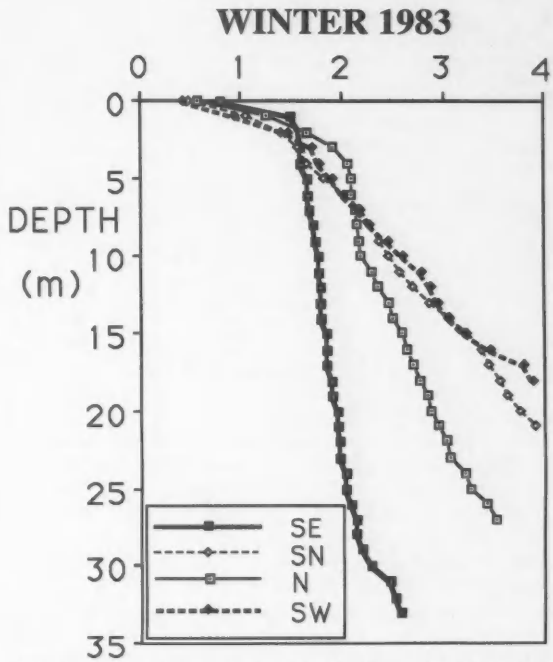

FIG. 5. Average winter temperature profiles from each basin for 1983. Each profile is based on an average from four soundings from early January to late March.

Winter

Stratification

Following freezeup water temperatures below the ice steadily increased. The rate of temperature change differed among basins with the SW Basin warming most rapidly and the SE Basin remaining the coolest (Fig. 5). Factors which cause the observed differences in temperature are similar to those for summer: basin specific factors such as morphometry, differential penetration of solar radiation, and differential groundwater inputs. Interbasin exchange would again reduce the differences.

Morphometric effects — To determine if differences in basin morphometry are the primary cause of the observed differences in temperature, an average winter heat flux was determined from early January to late March. This period does not include times immediately after freezeup or just prior to breakup, when heating from solar radiation can dominate (Scott 1964). For this period only 1983 and 1984 had sufficient data to compute mean heat fluxes. The average total heat fluxes into the entire water column varied only slightly among basins (Table 5), with the average from both years being 9.9, 7.8, 8.4, and 6.7 $cal \cdot cm^{-2} \cdot d^{-1}$ in the SE, SN, N, and SW Basins, respectively. Thus, a similar amount of heat being distributed into four different relative volumes does explain most of the differences in temperature. The relative volume of the entire water column is represented by the overall mean depth of each basin (Table

TABLE 5. Average winter heat flux ($cal \cdot cm^{-2} \cdot d^{-1}$) comparison: total into water column and below 6.5 m. Seasonal values are computed from profiles taken between the beginning of January and the end of March.

		Basin			
Year	Depth	SE	SN	N	SW
1983	Surface	11.1	9.3	7.3	7.0
	6.5 m	8.8	5.8	5.2	4.5
1984	Surface	8.5	6.3	9.5	6.3
	6.5 m	7.2	6.9	7.6	4.8
Average	Surface	9.9	7.8	8.4	6.7
	6.5 m	8.0	6.4	6.4	4.6

1). Therefore, the SW Basin, with the shallowest mean depth of 8.3 m, warmed most rapidly and the SE Basin, with the deepest mean depth of 17.2 m, warmed most slowly (Fig. 5).

Only weak stratification existed below 3 m (Fig. 5). Even though stratification was weak at the sill depths, isolation below the sills might be expected owing to the absence of external mixing forces because of the ice cover. The average heat fluxes into the volumes below the sill depths, i.e. around 6.5 m, were 8.0, 6.4, and 4.6 $cal \cdot cm^{-2} \cdot d^{-1}$ in the SE, SN, N, and SW Basins, respectively. Part of this variability may be explained by interbasin exchange. As winter progressed, basin temperatures at all depths became increasingly different and corresponding horizontal density differences developed (Fig. 2c). In the absence of surface wind stress, these density differences, although weak, would allow warmer, heavier water above the sills in warmer basins, e.g. SW Basin, to flow into and downward in the cooler basins, e.g. SE Basin. Cooler, lighter water in the deeper basin would then be displaced upward and move horizontally into the shallower basin. This water movement would result in the wedged-shaped thermal structure (Fig. 2c). The deeper (near 4°C) water in the shallower basins was hydrostatically stable and hence remained isolated. Interbasin exchange resulted in a net heat flux into the SE Basin and possibly into the N Basin and a net heat flux out of the SW and SN Basins. Heat transfer by interbasin exchange appeared to be significant but was not possible to quantify.

External effects — Solar radiation has been shown by Scott (1964) to have a minimal effect on the heat budget of Trout Lake during this winter period, since snow and ice reflect most solar radiation. However, solar radiation can be the primary source of heat during early and late ice covered periods.

Groundwater primarily infiltrates into Trout Lake along the southeast shoreline of the lake (Okweuze 1983; Kenoyer 1986). Groundwater apparently had a significant effect on the lake's thermal structure which can be seen by the sloping isotherms present on the east side of the SE Basin and not present on the west side of the SW Basin (Fig. 2c). Groundwater accounted in part for the larger heat flux into the SE Basin. Without knowing the volume of groundwater input, however, it is difficult to quantify its effect.

In winter, differences in temperatures among basins were primarily due to differences in basin morphometry or relative volumes. However, interbasin exchange and groundwater inputs also contributed to the observed thermal differences.

Conclusion

During summer stratification in Trout Lake, epilimnetic conditions were horizontally uniform, or in a sense, controlled or held constant and the variations in hypolimnetic temperatures were primarily due to morphometric differences among basins after correcting for variations in thermocline depths. Interbasin exchange contributed to the variations observed in thermocline depths and temperature gradients. During winter, the observed variations in temperature among basins were due to differences in morphometry, but also interbasin exchange and groundwater inputs.

Because variations in summer hypolimnion temperatures and winter temperatures were principally due to morphometry, this multibasin lake can provide the opportunity to test for morphometric effects in other models, such as areal hypolimnetic oxygen depletion models. The separate hypolimnia also can be used to replicate tests of various biological experiments without the confounding effects of differences in other climatological, edaphic, biological, and chemical factors.

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Performance of Triploid Pacific Oysters, *Crassostrea gigas*: Gametogenesis[1]

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Triploid Pacific oysters, *Crassostrea gigas*, were produced by treating newly fertilized eggs with cytochalasin B. After a year of growth, diploid and triploid oysters were sampled throughout the reproductive season to determine the effect of triploidy on gamete formation and sexual expression. Relative to diploids, gametogenesis in triploids was retarded but not absent: both male and female triploids produced some gonad. Gametogenesis in most triploid males resulted in spermatid production; all triploid males produced numerous spermatocytes. Oocyte formation in most triploid females was severely retarded, although some triploid females produced numerous eggs. Hermaphroditism in the triploid population was markedly higher than in diploids: 29 versus 1%, respectively. The ratio of males:females was the same in diploids and triploids.

On a obtenu des huîtres creuses du Pacifique, *Crassostrea gigas*, triploïdes en traitant des oeufs récemment fertilisés à la cytochalasine B. Après une année de croissance, on a recueilli des huîtres diploïdes et triploïdes pendant toute la saison de reproduction afin de déterminer l'incidence de la triploidie sur la formation des gamètes et l'expression des gènes sexuels. Par rapport aux diploïdes, la gametogénèse chez les triploïdes était retardée mais non absente : les mâles et femelles triploïdes ont produit quelques tissus reproducteurs. Chez la plupart des mâles triploïdes, des spermatides sont issus de la gametogénèse tandis que tous les mâles triploïdes ont produit de nombreux spermatocytes. Chez la plupart des femelles triploïdes, la formation d'ovocytes a été nettement retardée mais certaines femelles triploïdes ont produit de nombreux oeufs. Chez la population triploïde, l'hermaphrodisme était nettement plus marqué que chez les diploïdes : 29 % v. 1 %, respectivement. La proportion relative des sexes était la même chez les diploïdes et les triploïdes.

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Of the approximately 10 species of bivalves in which triploidy has been induced, histological descriptions of gametogenesis have been published for only two (Allen et al. 1986; Komaru and Wada 1989). In these two, gametogenesis is disfunctional. A close examination of the nature of the disfunction may lead to insights on mechanisms of normal function. For example, in triploid *Mya arenaria* gametogenesis is severely retarded; it also appears that all triploids are female (Allen et al. 1986), leading the authors to hypothesize a sex determining mechanism for this species.

Triploid *Mya* may not reflect the "typical" case in shellfish because triploids of other taxa (e.g. fish, amphibians) exhibit both sexes. In fact Allen and Downing (1986) demonstrated that triploid *Crassostrea gigas* include both males and females; males mature to a greater extent than females, based on relative cross-sectional area of gonad.

Gametogenesis in oysters has been extensively documented for diploids (Coe 1932; Kennedy and Battle 1963; Loosanoff 1942), including *C. gigas* (Katkansky and Sparks 1966; Berg 1969). Therefore, in this study we were interested in the differences between gametogenesis in diploid and triploid oysters, e.g. the time course of maturation, the nature and fate of gametes produced in triploids, and the manifestation of triploidy on sexual expression.

Materials and Methods

Production and Deployment of Triploids

Experimental triploids used in this analysis of gametogenesis were the same individuals for which overall growth, maturation, and glycogen content have been previously described (Allen and Downing 1986).

Briefly, the triploid population was produced by treating approximately 3 million newly fertilized eggs with 1 mg cytochalasin B (which had been dissolved in 1 mL of DMSO) in 1 L of sea water held at 20°C; treatment duration was from 30–45 min after fertilization. Untreated eggs from the same mass spawn served as the control diploid population.

Larvae were set on oyster shell cultch which was subsequently strung on long lines. Two long lines of triploids and one control were deployed in Humboldt Bay, California in June 1984. Beginning in May 1985, triploids and control diploids were sampled throughout their second reproductive season, at bi-weekly intervals in the summer and monthly in

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TABLE 1. Maturity in triploid hermaphrodites was described as mature or immature based on the maturity of the gametes in the follicles (as opposed to follicle or overall gonad size). The distinction was also made between those that apparently began gametogenesis as females or as males. In this way, four classes of hermaphrodites were described.

	Classification of Hermaphrodites Maturity	
	Immature	Mature
Coe (1932) classification	Functional	Morphological
Feminine		
Follicle structure	Growth and branching limited	Growth and branching limited
Gametes	Active auxocytes at base of follicle	Only mature eggs present
Masculine		
Follicle structure	Growth and branching extensive	Growth and branching extensive
Gametes	Spermatocytes differentiating along base of follicle wall	Spermatogenesis has reached maximum level attained in triploids

the fall. Growth was rapid in Humboldt Bay, so even though the experimental oysters were yearlings, tissue wet weights for whole oysters ranged from 10–30 g, or approximately 3–8 cm shell length at sampling (see Allen and Downing 1986).

Histological Preparations

Histological sections of whole oyster tissue were made from a section cut perpendicular to the anterior–posterior axis, just ventral to the labial palps (Allen and Downing 1986). Standard 5–7 μm paraffin imbedded sections were stained in hematoxylin and counterstained with eosin Y. For all individuals examined histologically, ploidy was determined using flow cytometry (Chaiton and Allen 1985) of adductor muscle (somatic) tissue.

Stages of Gonadal Maturation

Terminology for stages of gametogenesis used in this study are similar to those used by Allen et al. (1986) for *M. arenaria* and are compatible with those of Kennedy and Battle (1963) for *C. virginica*. Because gametogenesis in triploids is aberrant, gonadal maturation was assessed by assigning a state of maturity based on the kind, rather than degree, of gametogenesis. That is, gonads were scored by the types of inclusions in the follicles, but descriptions of relative numbers of cells, e.g. follicle half filled with spermatocytes, were not useful.

Criteria for Hermaphroditism

Hermaphrodites, i.e. the contemporaneous presence of male and female sex products, were observed in this study. Coe (1932) felt it was important to make a distinction between morphological and functional hermaphrodites. Morphological hermaphrodites are those individuals containing eggs and sperm in different stages of maturity. Coe considered morphological hermaphroditism to be a fortuitous byproduct of time of sampling. Functional hermaphroditism, on the other hand, represents a condition of simultaneously developing gametes, that presumably would be able to function when mature. This type of hermaphroditism is rare in natural populations.

In this study hermaphrodites were frequent in triploids. Because hermaphrodites were sampled during the period of gonad maturation, the distinction between morphological and functional hermaphroditism becomes less clear. Rather we distinguish between immature and mature hermaphrodites (Table 1): immature refers to hermaphrodites that have gametes of both sexes developing simultaneously (Coe's functional hermaphrodites), and mature hermaphrodites refer to those that have "mature" gametes from at least one of the sexes (Coe's morphological hermaphrodites).

We also made the distinction between hermaphrodites that (apparently) began gametogenesis as females (feminine) versus as males (masculine) (Table 1). This distinction was made by observing follicle structure, i.e. the amount of branching and growth, and the nature of the gonadal cells at the base of the follicle wall.

Results

Gametogenesis

Diploids: males and females

Maturation in diploids appeared normal. By the first sampling period (May 9), diploids were mostly in the middle active stage (Fig. 1). By June 22 both males and females were ripe and remained so until July 15.

Sometime between July 15 and August 12 (henceforth referred to as the spawning interval), diploids spawned. By September 11 most diploids were completely spawned out. Resorption was apparent in all diploids and was characterized by invasion of leucocytes in evacuated follicles. In the last sampling period, November 18, 8 of 15 diploids were still resorbing gametes; the remainder were beginning a new round of gametogenesis, with recognizable populations of oocytes or spermatocytes.

Triploids: males

Gametogenesis in triploid males was retarded relative to diploids (Fig. 1). In the first sampling period, the typical male gonad of triploids was categorized by active to middle active, with substantial populations of spermatocytes and (for middle active) some spermatids. In some cases, follicle branching was comparable with that in diploids, but follicle growth was apparently retarded, showing fewer spermatocytes and more undifferentiated spermatogonia than diploids of comparable maturity. In other cases follicles were only rudimentary and unbranched. The range of spermatid differentiation was from nearly none (active; Fig. 2) to extensive pockets of spermatids in most follicles (middle active; Fig. 3).

By May 23, when diploids were at a stage of late active, generally, the follicles of triploids seemed to have undergone little change. The proportion of spermatids in triploid follicles did not seem greater than on May 9, although all individuals had at least some. The "ripest" triploid was classified as late active (Fig. 4). A total of two males out of 38 sampled before August 12 reached late active. No triploid males matured beyond this stage.

Some triploid oysters spawned during the spawning interval for diploids: of 11 males sampled on August 12, four were spawned out and two had spawned partially. While we cannot prove it, it appears to us that spermatocytes still remained in

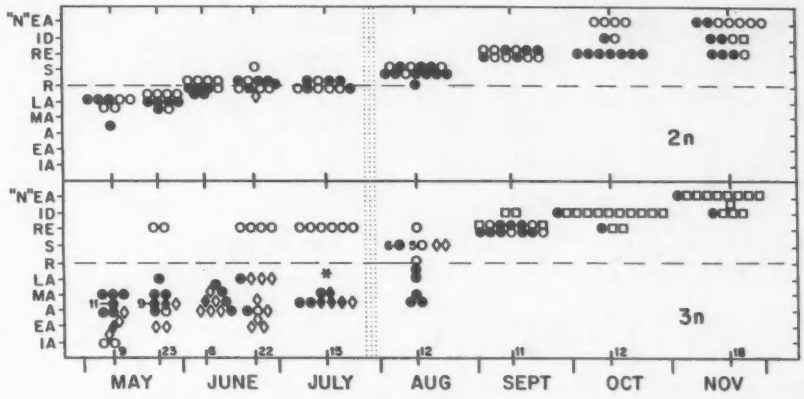


FIG. 1. Stage of maturation plotted by sample date. Males (closed circles), females (open circles), masculine hermaphrodites (closed diamonds), feminine hermaphrodites (open diamonds), and unknown sex (open squares) in diploid (2n) and tirploid (3n) *C. gigas* growth in Humboldt Bay. Asterisk is a diploid–triploid mosaic. Numerals before symbol indicate frequency of occurrence, e.g. 11 closed circles. Stippled region demarcates the pre-spawn samplings from the post-spawn ones: spawning for both diploids and triploids occurred between July 15 and Aug 12. Stages of maturity: IA = inactive; EA = early active; A = active; MA = middle active; LA = late active; R = ripe; S = spawned (partially or completely); RE = resorbing; ID = indifferent; "N"EA = "new" early active.

the gonoduct, representing cells that had been spawned, and were not new cell populations (Fig. 5). The remaining five were between active and late active. Individuals that had spawned partially or completely shared characteristics of histology with diploids: disorganized interfollicular tissue with enlarged (swollen) cells, collapsed follicles sometimes containing leucocytes, and gonoducts half empty of spermatids (or in the diploid case, spermatozoa). Unlike diploids, gonoducts of triploids that had spawned also contained spermatocytes. Completely spawned triploids must have evacuated many spermatocytes, in addition to spermatids.

In the September 11 sample, resorption was apparent in every male. By the last sampling date, nearly 75% of the triploids had commenced another cycle of gametogenesis (Fig. 6), but all but one was of unknown sex; by comparison, about half of the diploids sampled had begun gametogenesis anew.

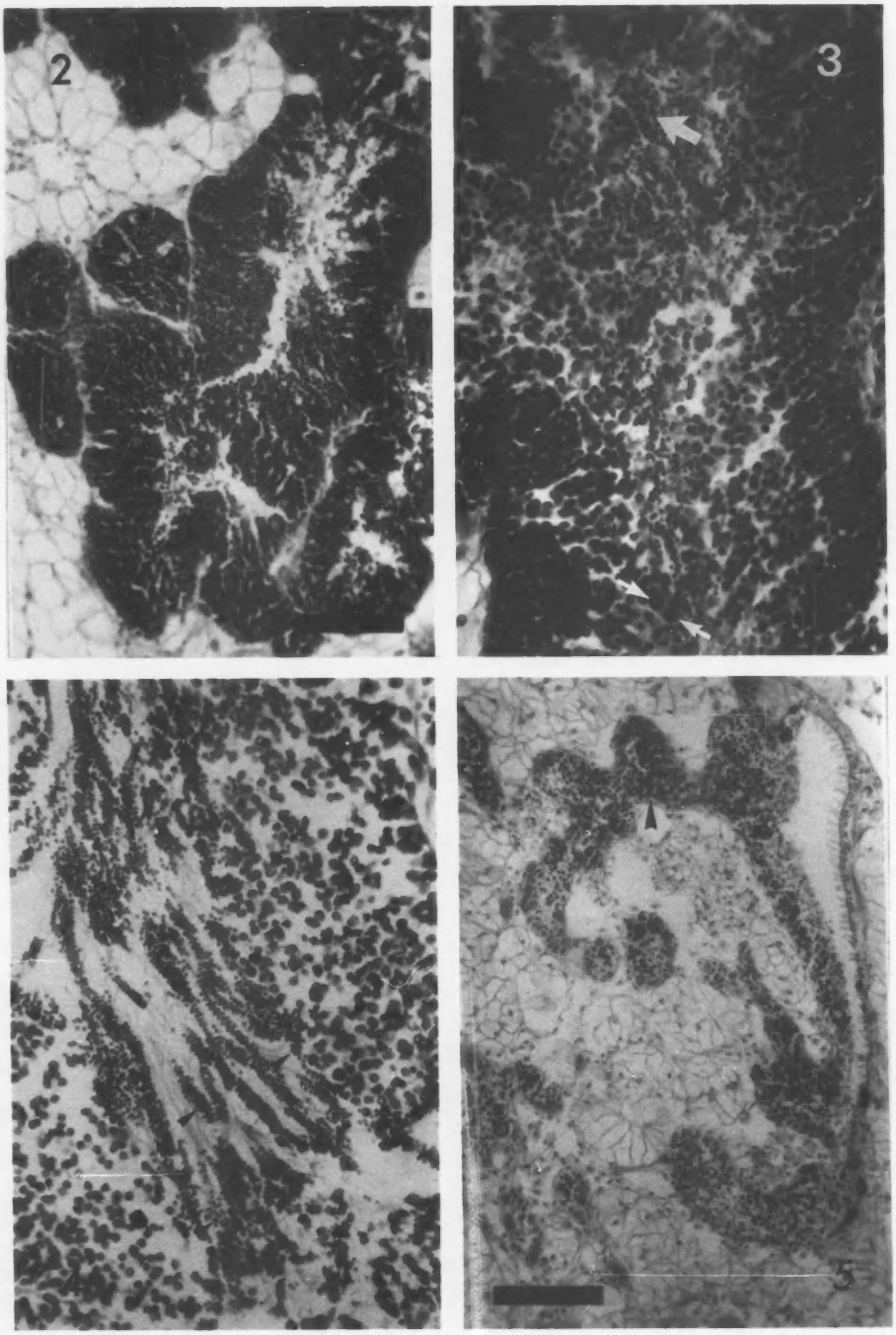
Triploids: females

Gonado- and gametogenesis in females was highly retarded, relative even to triploid males (Fig. 1). Typically, inactive female gonads showed little follicle branching and almost no follicle growth (Fig. 7). The lack of follicle branching was a characteristic of female histology used below to distinguish female from male hermaphrodites. Oogonia in the follicles were often abnormal. In abnormal oogonia, nuclei were pycnotic, and many were in zygotene stages, which suggested that they may have been arrested in this stage (Fig. 8). Some abnormal oogonia that had unusually clear cytoplasm appeared to be dead. An important character of the oogenesis in triploid females was a lack of developing oocytes. Females were identifiable because of the presence of ova in the follicles; however, for the most part ova were already fully developed, even in the first sampling period. Cells in female follicles tended to consist of arrested oogonia and mature ova (Fig. 9) that occurred in variable numbers from individual to individual. Most of the time the mature ova that were in the follicle were atretic or in the process of being resorbed, or both (hence most females were classified as resorbing in Fig. 1, even though they had been relatively inactive). Throughout the reproductive period, there was almost no change in activity in triploid females, so it appeared that gametogenesis had stopped very early in the reproductive season.

TABLE 2. Classification of hermaphrodites of *C. gigas* by type and sampling date. Hermaphrodites were classified as (I) immature or (M) mature (see text). Within these classes, the distinction was made between feminine and masculine hermaphrodites (also in text). Immature and mature hermaphrodites were tallied before and after the 06/06/85 sampling date, a date which seemed to naturally partition I and M types.

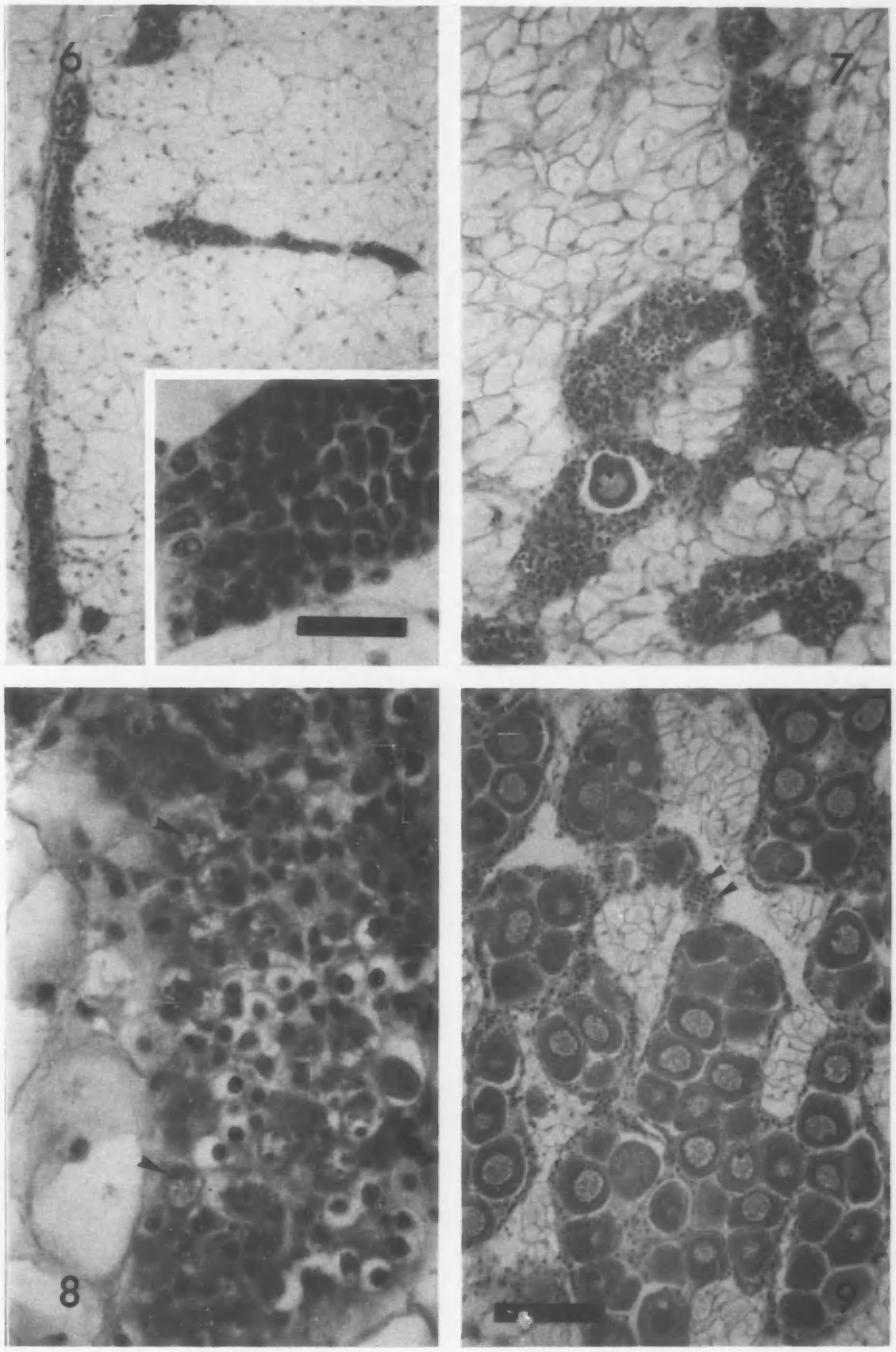
	Follicle type					
	Masculine		Feminine		Subtotals	
Sample date	I	M	I	M	I	M
05/09/85	1	1	2	—		
05/24/85	1	—	3	—		
06/06/85	1	—	4	1		
Subtotal	4		10		12	2
06/22/85	—	3	1	4		
07/15/85	—	4	1	—		
08/12/85	—	—	1	1		
Subtotals	7		8		3	12
Totals	11		18			

Some triploid females did produce relatively numerous ova (Fig. 10). Obviously, at some point they had gone through active stages of oocyte formation, but in these samples intermediate stages were not observed. Most triploid females appeared to have spawned during the spawning intervals (Fig. 11). No females were observed on October 12 on November 18.



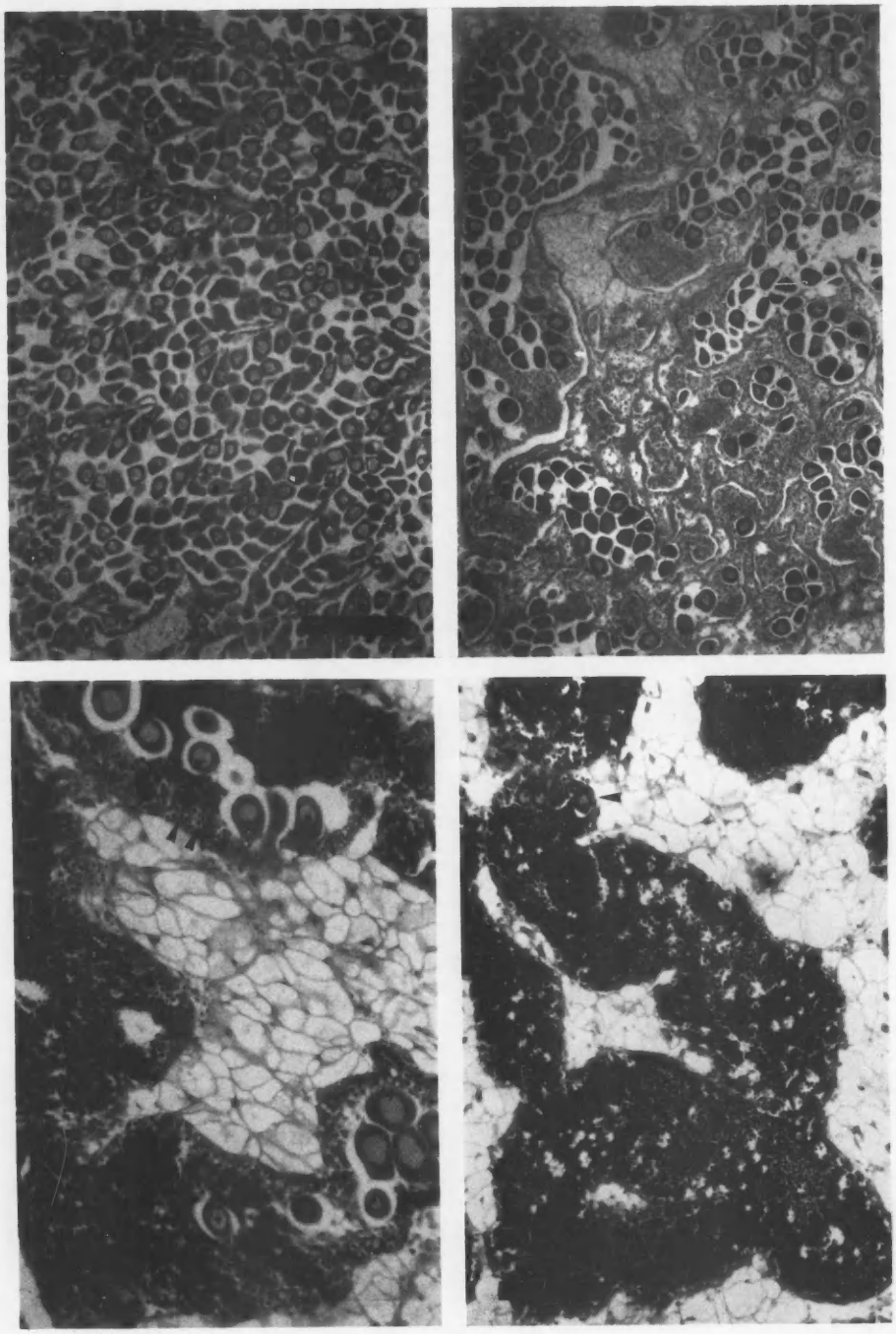
Figures 2–5

FIG. 2. Active stage of triploid *C. gigas* male. Similar in appearance to active diploids, triploids in this stage had large numbers of secondary spermatocytes, but no spermatid differentiation. Unlike diploids, however, this stage occurred late into the reproductive season, at a time when diploids were nearly mature. Note interstitial areas which had been absorbed by this time in diploids. Bar = 100 μm; 100×. FIG. 3. Middle active stage of triploid *C gigas* male. Triploid middle active stages were distinguished from active stages in this study because of the presence of spermatid (large arrow). Some multinucleate cells (small arrows) similar in appearance to those observed in abnormal spermatogenesis of *Mya arenaria* (Allen et al. 1986) were present. Bar (Fig. 2) = 40 μm; 250×. FIG. 4. Late active stage of triploid *C. gigas* male. Relative to other triploids, there were many mature cells in this individual; relative to diploids, gametogenesis is retarded despite good follicle growth. Both spermatids and spermatozoa (arrows) had differentiated. Bar (Fig. 5) = 40 μm; 250×. FIG. 5. Partially spawned stage of triploid *C. gigas* male. Similar to the diploid condition, there was tissue disorganization because of evacuated follicles. In general this disorganization was less severe because maturity was retarded and follicles were less full. Arrow shows spermatocytes still remaining after spawning. Bar = 100 μm; 100×.



Figures 6–9

FIG. 6. "New" early active stage of triploid *C. gigas* of unknown sex. Indifferent gonads began maturation by mitotic divisions of the gonia. Few triploids in this stage could be sexed. Bar = 100 μm; 100×. Inset is enlargement of gonial proliferation. Bar = 25 μm; 400×. FIG. 7. Inactive stage of triploid *C. gigas* female. The majority of females attained only this stage of development. Follicle branching and growth were underdeveloped. Much interstitial space remained as a result of this lack of development. Bar (Fig. 6) = 100 μm; 100×. FIG. 8. Oogonia of inactive triploid *C. gigas* female. Abnormal cells were found in inactive follicles. Some oogonia (arrows), apparently in a stage of leptonema–zygonema, were interspersed with cells with pycnotic nuclei and clear cytoplasms. When this individual was sampled, diploid females were between late active and ripe. But in triploids oocytes were not differentiating. Bar (Fig. 9) = 25 μm; 400×. FIG. 9. Ova of triploid *C. gigas* female. Oddly, many females had only "ripe" eggs and arrested oogonia, but few to no developing oocytes. Perhaps those eggs that could matured very early in the reproductive season, and those that could not remained arrested (note pycnotic nuclei among oogonia; arrows). Some eggs were being resorbed. Bar = 100 μm; 100×.



Figures 10–13

FIG. 10. Triploid *C. gigas* with many eggs. Some triploid females produced many ova of variable quality. Note large ova with dark staining nuclei, perhaps dead, and many developing oocytes still at follicle wall. Bar = 250 μm; 40×. FIG. 11. Spawned-out stage of triploid *C. gigas* female. In this individual nearly all follicles, except those shown here for effect, were empty of ova. Like their diploid counterparts, female triploids seemed to evacuate nearly all of their gametes. Interstitial tissue was disorganized, and presence of leucocytes indicated resorptive stages. Bar (Fig. 10) = 250 μm; 40×. FIG. 12. Feminine, immature hermaphrodite of triploid *C. gigas* in early development. Follicle structure of feminine hermaphrodites was similar to that of triploid females, with little branching or growth and cells with pycnotic nuclei (arrows). However, male gametogenesis was also in progress. Here developing eggs were growing concurrently with spermatocytes, the male portion best described as active. Bar (Fig. 13) = 100 μm; 100×. FIG. 13. Masculine, immature hermaphrodite of triploid *C. gigas*. Follicles are typically male-like in appearance early in development and spermatogenesis is obvious, except that oocytes are differentiating as well (arrow). Bar = 100 μm 100×.

TABLE 3. Distribution of sexes in diploid and triploid *C. gigas* sampled from Humboldt Bay, by sampling period. Parentheses = percents of diploids or triploids sampled, excluding indeterminate sexes.

	Diploid			Triploid		
Sample date	Male	Female	Hermaphrodite	Male	Female	Hermaphrodite
05/09/85	13 (76)	4 (24)	0	16 (73)	2 (9)	4 (18)
05/24/85	9 (64)	5 (34)	0	13 (65)	3 (15)	4 (20)
06/06/85	5 (45)	6 (55)	0	4 (40)	0	6 (60)
06/22/85	5 (45)	5 (45)	1(9)	2 (13)	5 (33)	8 (53)
07/15/85	6 (55)	5 (45)	0	3 (21)	6 (43)	5 (36)
08/12/85	10 (71)	4 (29)	0	11 (55)	7 (35)	2 (10)
Totals	48 (62)	29 (37)	1(1)	49 (48)	23 (23)	29 (29)

Hermaphrodites

Immature hermaphrodites constituted the majority in the first three sampling periods (Table 2) and most of these (9/12) were feminine: they were characterized by a typical triploid, female histology, except that spermatocytes and spermatids were present in the lumen of most follicles. Examination of several feminine types (Fig. 12) suggested that spermatocytes matured, but oocytes did not. Three of 12 immature hermaphrodites sampled in the first three periods were masculine. Spermatocytes dominated every follicle in masculine immature hermaphrodites (Fig. 13).

Mature hermaphrodites, about equal numbers of masculine and feminine types, dominated the mid-sampling periods (6/22–8/12, Table 2). The former were typical of late active, male triploids (Fig. 14), except that several to many ova (often atretic) were present. Feminine, mature hermaphrodites had typical female follicle development, and several to many mature ova were present; also, mature spermatids were observed in most follicles (Fig. 15a, b), and these were often abundant.

The transition from mostly immature to mostly mature hermaphrodites seemed to take place by the third sampling period (Table 2). Oysters taken from the first three periods were in the process of maturing and tended to have gonia of both sexes differentiating. The transition to maturity coincided with the period when diploids also attained maturity. Also, the numbers of hermaphrodites observed increased as the population of oysters grew more sexually mature, peaking in the June samples, when more than 50% of triploids were hermaphrodites.

One unique hermaphrodite was found among the samples taken. The somatic tissue sample (adductor muscle) of this individual had diploid and triploid cells, as determined by flow cytometry. Histologically, different regions of the gonad seemed to have matured in different ways, i.e. normal male (Fig. 16a), normal female (16b), and abnormal, so possibly triploid (Fig. 17).

Sex Ratio

Males, females, and hermaphrodites in diploids and triploids were tabulated for each sampling period (Table 3), until the sampling period when individuals of indeterminate sex appeared (September 11). In diploids, there was a uniform sex ratio throughout the first six sampling periods (null hypothesis: proportions of males: females were homogeneous; $G_H = 4.22$, $p > 0.50$). Over all sampling periods, the sex ratio in diploids was male 1.6:female 1.0. We observed one hermaphrodite among the diploids sampled.

For triploids, males and females were not distributed homogeneously over the initial six sampling periods ($G_H = 18.32$, $p < 0.01$). The proportion of males and females decreased in correlation with the increase in hermaphrodites. The sex ratio over all six sampling periods was male 2.1:female 1.0, which was not significantly different from the sex ratio of males to females in diploids ($G_H = 0.59$, $p > 0.50$). There was a significant difference between the ratio of males:females: hermaphrodites in diploids and in triploids ($G_H = 28.9$, $p < 0.001$, 2×3 contingency comparison). That is, the major distinguishing feature of the sex ratio in triploids was that hermaphrodites occurred more frequently. The ratio of triploid males, including masculine hermaphrodites:females, including feminine hermaphrodites (1.4:10) was not significantly different from the diploid sex ratio ($G_H = 0.00$, $p > 0.99$). However, the ratio of masculine hermaphrodites:feminine hermaphrodites (10:1.9) was not the same as that of diploid males: females ($G_H = 4.75$, $p < 0.05$) or that of triploid males:females ($G_H = 7.65$, $p > 0.01$). Hermaphrodites had more female types than would be predicted from the sex ratios of diploids or triploids.

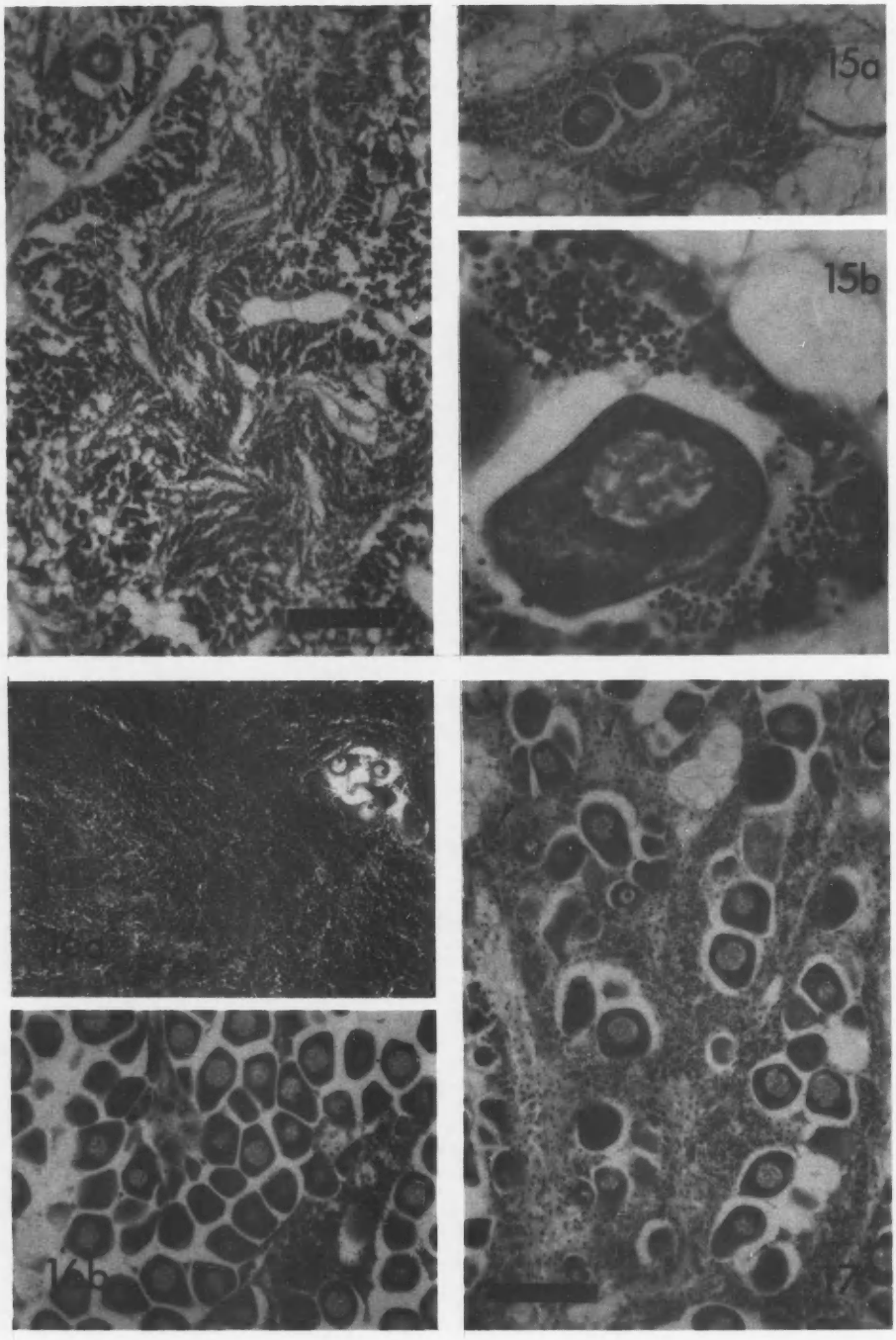
Discussion

Histology of Gametogenesis

Gametogenesis in diploids proceeded normally, judging from previous published accounts (Amemiya 1929; Quayle 1969). Gametogenesis in triploids ranged from somewhat abnormal to highly abnormal. "Ripe" follicles of triploid males were filled with spermatocytes, but often contained numerous spermatids; some spermatozoa were produced. Gametes produced by the more "mature" triploid females were numerous, although follicle development was retarded relative to the diploid females. But the typical case for triploid females was an abnormal and retarded state of gonado- and gametogenesis. The general state of gonadal development in triploid *C. gigas* was quite similar to that seen in triploid *C. virginica* by Lee (1988).

Chevassus (1983) divided sterility into three categories: "gonadic", "gametic", and "zygotic". Gonadic sterility is the condition where gonads do not develope; examples include several amphibian species (Fankhauser 1945) and, in some cases, triploid *Mya arenaria* (Allen et al. 1986). Zygotic sterility is obtained when gametes are viable and fertilization occurs, but inviable larvae or other early abortion results. Zygotic sterility occurs in triploid male rainbow trout (Lincoln and Scott 1983). Finally, triploids of most fish species are gametically sterile: gametes are frequently abnormal and scarce (Thorgaard 1983).

Triploid Pacific oysters cannot be categorized by any of these classes. Substantial gonads develop in males so sterility is not



Figures 14–17

FIG. 14. Masculine, mature hermaphrodite of triploid *C. gigas*. Except for the presence of ovum (which was atretic, arrow), this would have been classified as a late active triploid male. Bar = 100 μm; 100×. FIG. 15. (a) Feminine, mature hermaphrodite of triploid *C. gigas*. Only mature gametes of each sex were present in a female-type follicle. Bar (Fig. 14) = 100 μm; 100×. (b) An enlargement of a portion of follicle demonstrating ripe ovum and mature spermatids side by side. Bar (Fig. 14) = 25 μm; 400×. FIG. 16 (a) Diploid–triploid mosaic hermaphrodite of *C. gigas*, normal male section. Sperm occupied all follicle areas. Bar (Fig. 17) = 100 μm; 100×. (b) Diploid–triploid mosaic hermaphrodite of *C. gigas*, normal female section. Ova occupy all follicle areas. Bar (Fig. 17) = 100 μm; 100×. FIG. 17. Diploid–triploid mosaic hermaphrodite of *C. gigas*, abnormal (triploid?) section. Eggs compared with those in other sections appeared less developed and several were in resorption. Some pycnotic-type nuclei can be seen (arrows). Bar = 100 μm; 100×.

"gonadic". Gametes are produced by both males and females, and some of the spermatozoa are normal and produce normal (i.e. diploid) embryos (S. K. Allen, Jr. and S. L. Downing, unpubl. data) so sterility is not "zygotic" or "gametic".

Maturation in triploid males is relatively uniform. At least, all triploid males are capable of significant primary spermatocyte production, and most are capable of secondary spermatocyte (meiosis I) and spermatid (meiosis II) production. Although there is some variation in the number of spermatids produced, it seems less variable than egg production among female triploids. Perhaps gamete maturation in triploid male oysters is simply a function of the ability of sister chromatids to segregate, not necessarily faithfully. That is, non-disjunction of chromosomes or chromosome pairs (because of pairing abnormalities of triploids) might slow, but not prevent, meiosis.

For triploid females, egg production in those that "mature" stands in sharp contrast to the state of maturity in triploid, female finfish (Thorgaard 1983). In these individuals there was nearly complete retardation of any gonado- or gametogenesis. Some triploid female *Mya arenaria* also developed large numbers of ova (Allen et al. 1986). For the most part, however, triploid females mature very little. Oyster eggs, and those of molluscs in general, do not have to complete any meiotic maturation divisions before oocyte growth (Longo 1983). Therefore it seems unlikely that sterility in triploid females is related to problems with pairing of sister chromatids.

Despite abnormal gametogenesis in triploids they were able to spawn. Both males and females showed unmistakable signs of having spawned between July 15 and August 12, in correspondence to the period when diploids also spawned. This was interesting because the gonads of triploids were not fully matured. It appeared, based on residual cell masses left behind in the triploid gonoduct, that spermatocytes and spermatids were spawned along with whatever spermatozoa may have been present. We question how complete the feedback is between the ripe or ripening gonads and the physiological cues that promote the behavior of spawning.

Hermaphrodites

One of the outstanding features of sexual maturity in triploid *C. gigas* was the incidence of hermaphrodites. Twenty-nine percent of triploids sampled were hermaphroditic. This compared with 1% incidence among diploids sampled. Oviparous oysters in general are classified as alternative sexuality hermaphrodites (or sequential hermaphrodites (Hoagland 1984)) because they function seasonally as separate sexes (Coe 1943). However, the frequency of hermaphrodites during a given reproductive season in *Crassostrea* spp. is very low in nature. For *C. virginica*, approximately 1% (Burkenroad 1931; Coe 1943) occurred as hermaphrodites; for *C. gigas*, between 1 and 3% were hermaphroditic (Katkansky and Sparks 1966).

Coe (1943) defined accidental (or developmental) hermaphroditism as "...resulting from deviations in the developmental processes due to the failure of the sex differentiating mechanism to function normally". Both Coe (1943) and Purchon (1968) suggest that there is a delicate balance between the expression of the gonochoristic and hermaphroditic states. In fact, the primary gonad of *C. virginica*, and probably those of other alternative sexuality hermaphrodites, usually contains the antecedent cells of both sexes (Coe 1943). Coe likens the variability in sexuality of different individuals, and in some cases in a single individual at different periods of life, to the variability in color patterns of certain mammals or plants. Both may be phenotypes of various multiple allele combinations.

A multiple-allele-sex determining system has been proposed by Haley (1977). His two allele (male and female), three locus (the minimum number needed to explain his data) model would act in an additive fashion. Individuals with three or more male alleles are male, which requires three heterozygous loci or at least one fixed locus; individuals with five or six female alleles (at least two of three loci fixed) are females. Increased heterozygosity favors maleness. Under the multiple allele model, we would expect the sex ratio to shift toward males with increased heterozygosity in triploids (see Allendorf and Leary 1984). However, the sex ratios of males to females in diploid and triploid groups were the same.

Probably, hermaphrodites do not occur as a discrete class like male or female, but occur at the expense of males and females. This is indicated by the fact that hermaphrodites did not occur uniformly throughout the sampling season; they occurred more frequently as maturity reached its peak. If they were a distinct class, hermaphrodites would have been distributed more uniformly. It seems that while both males and females are capable of producing female or male gametes, respectively, females are more likely to produce sperm than males are to produce eggs, as suggested by the ratio of about two female hermaphrodites to one male hermaphrodite.

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Estimating the Blubber Content of Phocid Seals

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We have investigated the relationships between percent blubber content and xiphosternal blubber thickness or girth-to-length ratios in ringed seal (*Phoca hispida*), harp seal (*Phoca groenlandica*), and grey seal (*Halichoerus grypus*). The blubber content was significantly correlated with blubber depths and girth-to-length ratios in all three species. In addition, we have developed an estimator for percent blubber content (the LMD-index) based on standard length (*L*, in meters), body mass (*M*, in kilograms), and on blubber thickness (*d*, in meters) measured at a defined position dorsally. From these variables the percent blubber content (B%) can be estimated by the expression $B\% = 4.44 + 5693\ \sqrt{L/M \cdot d}$, with a standard error of the estimate of three percentage units. This index also gave reasonable estimates for blubber content in three harbour seals (*Phoca vitulina*) and eight bearded seals (*Erignatus barbatus*).

On a étudié les relations entre le volume de petit lard (en pourcentage) et l'épaisseur du petit lard de l'appendice xiphoïde ou les rapports entre le volume de l'abdomen et la longueur du phoque annelé (*Phoca hispida*), du phoque du Groenland (*Phoca groenlandica*) et du phoque gris (*Halichoerus grypus*). Le volume de petit lard était nettement lié à son épaisseur et au rapport entre le volume de l'abdomen et la longueur du corps chez ces trois espèces. De plus, on a calculé un estimateur du volume de petit lard en pourcentage (l'indice LMD) basé sur la longueur standard (L, en mètre), la masse corporelle (M, en kg) et l'épaisseur du petit lard (d, en mètre) mesurée à un point précis sur le dos. En se basant sur ces variables, on peut déterminer le volume de petit lard en pourcentage (B %) à l'aide de la formule $B\% = 4{,}44 + 5693\ L/M.d$ avec un écart-type de l'estimation de trois unités de pourcentage. Cet indice donne aussi des estimations acceptables du volume de petit lard de trois phoques communs (*Phoca vitulina*) et de huit phoques barbus (*Erignatus barbatus*).

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Because heat loss to water is many times higher than to air, aquatic life poses special problems for homeothermic animals. In cetaceans and phocid seals, the intra- and subdermal fat tissues, called the blubber, forms the main protection against heat loss. In addition, the blubber fat serves as an energy reserve, although the relative importance of fat stored in the blubber and in the core (muscle, skeleton, and viscera) varies between species (Worthy and Lavigne 1987). The fat stores probably influence fertility in marine mammals (Lockyer 1986; Smith 1987; Testa 1987), as they do in terrestrial mammals (Hamilton and Blaxter 1980; Thomas 1982), and fat content varies throughout the year in conjunction with the reproductive cycle (Fedak and Anderson 1987; Ryg et al. 1990).

As a consequence, there is great interest in methods for determining body condition of marine mammals. Direct measurement of blubber or fat mass by dissection or extraction of fat from the whole body is laborious, and it is common to use some kind of body condition index to evaluate nutritional condition. The two indicators most commonly used for seals are the xiphosternal blubber thickness and the "condition index"; maximum girth divided by standard length times 100 (Sergeant 1973).

The extent to which these indices are related to blubber content have not been quantitatively assessed. They appear to be relatively insensitive, even failing to pick out individuals that are, from inspection, close to death from starvation (T. G. Smith, pers. observ.). They also appear to be only weakly correlated with each other (Pitcher 1986). Better indices of body condition are therefore needed.

Here we examine the relations between percentage blubber content and the xiphosternal blubber thickness, and the girth/length ratio. We also develop a new index for calculating blubber content. This "LMD-index" is calculated from the following variables: standard length (*L*), body mass (*M*), and blubber thickness (*d*)). Using data from five seal species, we suggest that the LMD-index can be used to estimate blubber mass for phocid seals in general.

Materials and Methods

Collection of Data

Five species of seals were considered in the analysis. One hundred and thirty two ringed seals (*Phoca hispida*) were shot from the end of February to mid September in three consecutive years (1985 to 1987), 19 from the Barents sea and the rest from Kongsfjorden (78°55′N, 11°58′E) on Svalbard, Norway. Eight bearded seals (*Erignatus barbatus*) were shot in Kongsfjorden in May 1989. Thirty-eight grey seals (*Halichoerus grypus*) were shot on Anticosti Island in the Gulf of St. Lawrence, Canada from July to August 1987. Twenty harp seals (*Phoca groenlandica*) that had drowned in fishing nets on the northwest coast of Norway were collected in January and February 1987. Three harbour seals (*Phoca vitulina*) which had died during the North Sea epizootic in 1988 (Osterhaus and Vedder 1988) were also included in the total sample (the actual cause of death of these individuals was not confirmed).

The animals were weighed, and standard measurements taken (American Society of Mammalogists 1967). Weighing of seals was done with spring balances to the nearest 100 g. The standard length (nose-to-tail length in a straight line) of the seals should ideally be measured before rigor sets in. The dead seals were placed belly upwards, and pulled gently by their hindlimbs to stretch them out. In this way one avoids either having the neck pulled into an *S*-shape, or overstretching the neck. In seals which had been frozen, we often had to forcibly straighten out the neck, which may have introduced errors in the length measurements. Girths were measured around the snout and head and every 10 cm behind the snout, back to just in front of the anus.

The sculp (skin with attached blubber from body and head, but excluding flippers) was carefully dissected from the body core, placed on a flat surface, and stretched out so that sculp length was equal to the standard length of the animal. The blubber thickness was measured at every 10 cm across the length and width of the sculp. Thickness measurements did not include the skin. Blubber mass was determined by subtracting skin mass from sculp mass, after thoroughly removing the blubber from the skin.

Data Analysis

Blubber content, as percent of body mass, was compared with the classical indices of body condition, xiphosternal blubber thickness, and "condition index," 100 × maximum girth/standard body length of the harp seals, grey seals, and ringed seals. The bearded and harbour seals were not used in these comparisons because of the small sample sizes.

Blubber content was also compared with the expression $\sqrt{L/M}\cdot d$, where L is standard body length in metres, M is body mass in kilogramd and d is dorsal blubber thickness in metres, measured at a standard position defined below. The rationale for testing this relationship was that the volume of blubber is equal to $B = A\cdot d_{mean}$, where A is axial surface area and d_{mean} is mean blubber thickness. Ryg et al. (1988) showed that the axial surface area can be expressed as $A = 0.112\cdot\sqrt{M\cdot L}$, and that the blubber thicknesses at various parts on the body are linearly related to each other. Blubber volume can thus be expressed as Blubber $= k\cdot\sqrt{L\cdot M\cdot d}$, where the proportionality constant k depends upon which blubber thickness is chosen and the density of the blubber. Dividing by body mass gives the percentage blubber content: $B\% = 100k\cdot\sqrt{L/M\cdot d}$. Because of the small sample sizes, the relations between blubber content and LMD-index were not calculated for bearded and harbour seals. Instead, measured blubber content was compared with the fat content predicted on the basis of the relation between blubber content and $\sqrt{L/M\cdot d}$ derived from the other three species.

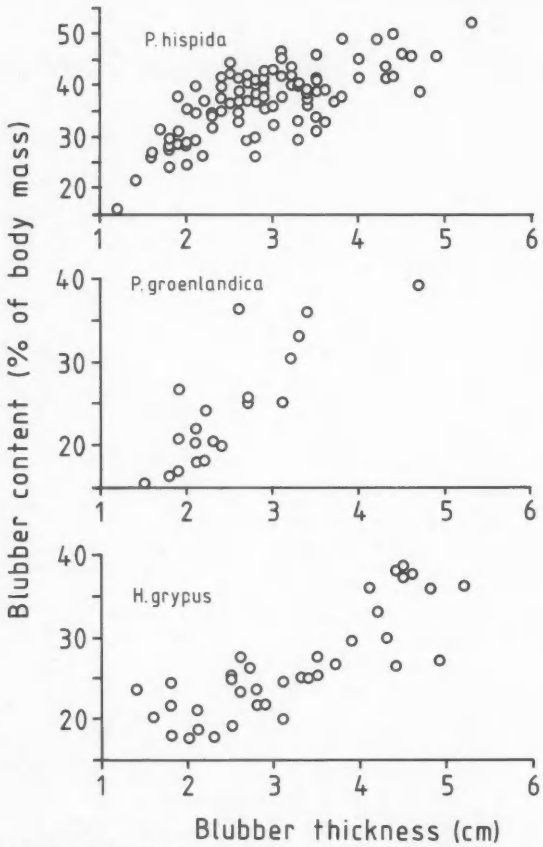

FIG. 1. Relationship between percentage blubber content and sternum blubber thickness.

The position of choice to measure the blubber thickness is where it is most variable, following the recommendation of Lockyer et al. (1985) for whales. Ryg et al. (1988) found that the most variable location of blubber thickness in ringed seals is dorsally, about 60% of the standard body length behind the snout. The blubber thickness at this position (found by interpolating between the nearest points on the 10 × 10 cm grid) was used in the expression above.

Least squares linear regression analysis was used to find the relationship between the various indices and the blubber content. Differences between the slopes and elevations of regression lines were tested by analysis of covariance (Snedecor and Cochran 1967). Other differences were tested with student's *t*-test.

Results

The blubber content, expressed as percent of total body weight, was significantly correlated ($p<0.01$) with xiphoster-

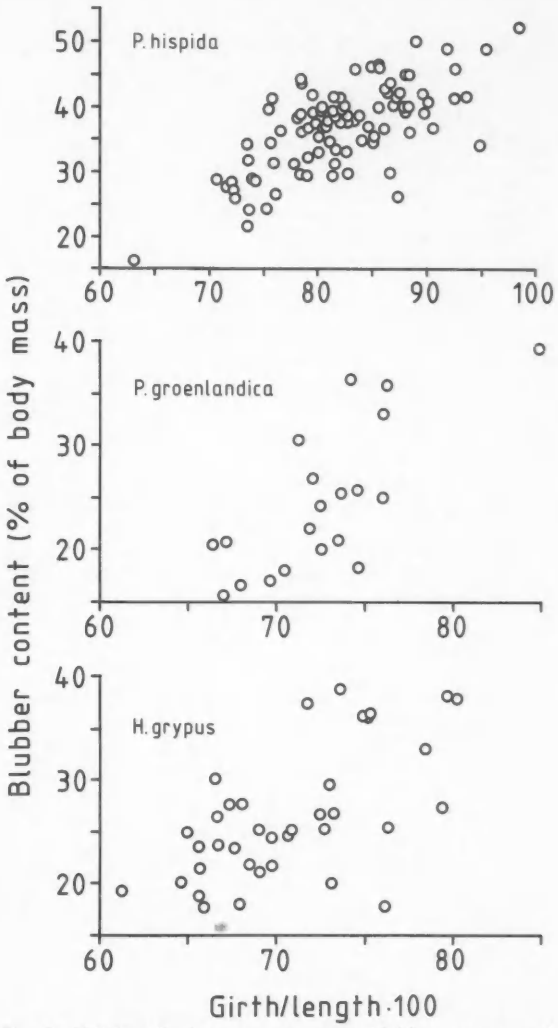

FIG. 2. Relationship between percentage blubber content and 100·girth/length.

nal blubber depth. (Fig. 1). The regressions between percentage blubber content and blubber depth were for ringed, harp, and grey seals:

P. hispida
Percent blubber = $551 \cdot d + 22.0$ $r^2 = 0.46^{**}$ $S_{xy} = 4.85$

P. groenlandica
Percent blubber = $807 \cdot d + 4.35$ $r^2 = 0.70^{**}$ $S_{xy} = 4.01$

H. grypus
Percent blubber = $497 \cdot d + 10.7$ $r^2 = 0.69^{**}$ $S_{xy} = 3.64$

The percentage blubber content was significantly correlated ($p<0.01$) with the condition index (100 × girth/length) (Fig. 2). The regressions between percentage blubber content and condition index were for ringed, harp, and grey seals:

P. hispida
Percent blubber = $0.66 \cdot$condition index $- 16.29$ $r^2 = 0.39$ $S_{xy} = 5.17$

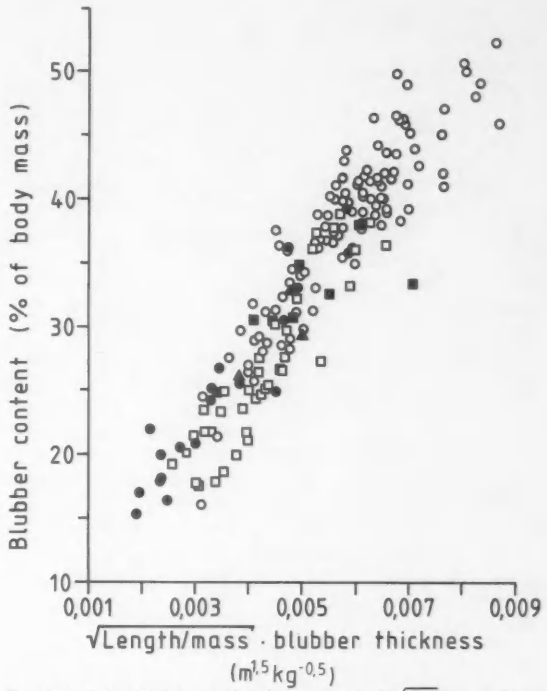

FIG. 3. Relationship between blubber content and $\sqrt{L/M} \cdot d$. ○ = Phoca hispida, ● = Phoca groenlandica, □ = Halichoerus grypus, ■ = Erignatus barbatus, ▲ = Phoca vitulina.

P. groenlandica
Percent blubber = $1.27 \cdot$condition index $- 67.31$ $r^2 = 0.55$ $S_{xy} = 4.91$

H. grypus
Percent blubber = $0.83 \cdot$condition index $+ 32.13$ $r^2 = 0.38$ $S_{xy} = 5.10$

Condition indices and xiphosternal blubber thicknesses were significantly correlated in all three species. The correlation coefficients were 0.71, 0.83, and 0.59 for grey, harp, and ringed seals, respectively.

Percentage blubber content and the expression $\sqrt{L/M} \cdot d$ were significantly correlated ($p<0.01$, Fig. 3). The regressions between percentage blubber content and $\sqrt{L/M} \cdot d$ were for ringed, harp, and grey seal:

P. hispida
Percent blubber = $5102 \cdot \sqrt{L/M} \cdot d + 8.53$ $r^2 = 0.82$ $S_{xy} = 2.80$

P. groenlandica
Percent blubber = $5387 \cdot \sqrt{L/M} \cdot d + 6.13$ $r^2 = 0.90$ $S_{xy} = 2.35$

H. grypus
Percent blubber = $5650 \cdot \sqrt{L/M} \cdot d + 2.27$ $r^2 = 0.83$ $S_{xy} = 2.68$

The slopes and elevations of the regression lines for harp, ringed, and grey seal species (Fig. 1) were not significantly different ($0.1>p>0.05$). For the pooled sample the regression equation was:

Percent blubber = $5693 \cdot \sqrt{L/M} \cdot d + 4.44$ $r^2 = 0.88$ $S_{xy} = 3.02$

In eight bearded seals, with blubber content ranging from 25 to 38% of body mass, observed blubber mass was not significantly different from the blubber mass estimated from the equation above (paired t-test, $t = 0.74$). With one exception, where

the observed blubber content was 11 percentage units lower than estimated, the difference ranged from −2.88 to 3.15 percentage units. The "exceptional" animal also had a very high condition index in relation to its actual blubber content, and we cannot exclude the possibility of an error in data recording.

In three harbour seals, the estimated and observed blubber content were 31.6 and 33.0; 26.2 and 26.1; and 29.5 and 32.9, respectively. The difference was not statistically significant.

The positive y-intercept was significantly different from zero for all species except grey seal, and also for the pooled sample (Fig. 3).

Discussion

Knowledge of the mass of the blubber permits calculation of the minimum heat loss through the body surface (Ryg et al. 1988), and consequently gives a lower limit to the metabolic rate needed to maintain homeothermy. Provided the fat content of the blubber is known, it also gives an estimate of the energy reserves contained in the blubber. However, nutritional condition depends on more than the energy content of the blubber layer. Fat may also be stored in the core (i.e. muscles and viscera) (Worthy and Lavigne 1987), and to evaluate the condition one must also consider the normal seasonal fluctuations in body weight and body fat content.

Blubber thickness, the condition index, and our proposed LMD-index were all correlated with percent blubber mass. The relationship between blubber content and xiphosternal blubber thickness was different for the three species investigated, as a given blubber thickness was related to a lower blubber content in the grey seal than in the other seals. This is to be expected, as a given blubber thickness will constitute a smaller proportion of the body radius in larger animals. The LMD-index was a more precise estimator than the other two, provided a linear estimator of percentage fat content, and was species-independent as far as we have been able to ascertain. In contrast, the condition index probably reflects differences in the volume of the core as well as the blubber layer. Use of both indices could help to elucidate patterns of fat loss from blubber and core.

It is important to be aware of the limitations in use of indices. The S_{xy} of 3% of the error of blubber mass prediction from the LMD-index gives a confidence interval for individual predictions of about 6%. This corresponds to a confidence interval of individual heat loss estimates of ±28% at 20% blubber content, and ±15% at 50% blubber content (Ryg et al. 1988). One should also be careful not to use the LMD-index uncritically outside the range where it has been verified empirically. The value of the index must necessarily be zero for zero blubber content (since the blubber thickness then is zero), and the positive y-intercept therefore means that the line must curve downwards for low values. We do not know the reason for this nonlinearity at low values of blubber content, but it could be caused by nonlinear relations between blubber thickness in very lean seals. Our arguments for the LMD-index is that blubber thicknesses at different locations of the body are proportional, but this may not be the case outside the range of blubber contents that are found in our material.

The statistical error of the LMD-index depends on the errors in measurements of length, mass, and blubber thickness, and can be calculated by the expression

$$S_{LMD}/LMD = \sqrt{(0.5 \cdot S_M/M)^2 + (0.5 \cdot S_L/L)^2 + (S_d/d)^2}$$

where S_M, S_L, and S_d are the standard deviations of the mass, length, and thickness measurements (Bevington 1969). Length measurements do not require more than an ordinary tape; however the standardization of the measurement is important (American Society of Mammalogists 1967). If quality weighing equipment is available, the body mass can be determined with an accuracy of about 1%, even under field conditions. The index gives the blubber in percent of the mass as measured, and one should avoid correcting for blood loss before the index is calculated. Variations in the amount of blood loss will therefore introduce errors in the estimate of the blubber content of the live animal.

According to the expression above the error in the blubber thickness measurement contributes four times more than error in weight and length determinations. Since it should be possible to bring the variation coefficients for mass and length down to about 1%, and it is difficult to measure the usual range of 1 to 6 cm blubber thickness with any greater accuracy than 1 mm, the statistical error in the index will be dominated by the error in measurement of blubber thickness.

If the seal dissections are part of a commercial venture, measuring the dorsal blubber thickness may represent a problem because one will ruin the skin if it is cut in that position. It is therefore desirable to develop a simple and rapid method for measuring the dorsal blubber thickness of seals without ruining the skin. Non-intrusive methods could possibly also be used on live animals.

In conclusion, we have found that the two classical indices of condition in phocid seals, xiphosternal blubber thickness, and girth-to-length ratio, both are positively correlated with blubber mass as percent of body mass. We have also developed an index based on length, body mass, and dorsal blubber thickness, which can be used to estimate percent blubber mass of phocid seals in general.

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REVIEW/SYNTHÈSE

Effectiveness of Crab and Lobster Traps

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Traps are a versatile and powerful fishing gear. Desired species and sizes can be targeted through trap design and the choice of bait. Size of the catch is affected by trap size, bait quantity and quality, time between setting and hauling, and preventing escape through the entrance. But the largest potential for increasing trap catches is by increasing ease of entry and reducing the effect of gear saturation (animals inside traps preventing those outside from entering). Using catch per trap as an index of abundance is attractive for both fisheries management and ecological studies. However, correlations between catch and abundance have not been well established, probably because of the many factors affecting catchability (e.g. stage of the molt and reproductive cycles, sex, animal size, lunar and diurnal cycles, temperature, and water motion). Methods for conducting trapping surveys, measuring catchability, and comparing fishing strategies are critically reviewed.

Les casiers constituent un engin de pêche polyvalent et efficace. Leur conception et le choix de l'appât permettent de capturer des espèces cibles et de la taille souhaitée. La dimension des casiers, la quantité et la qualité des appâts, le temps écoulé entre la mise à l'eau et le halage, et la prévention de leur fuite par l'ouverture influent sur la taille des prises. Mais les meilleures chances d'augmenter les prises au moyen de casiers consistent à faciliter davantage l'entrée et à réduire l'effet de saturation de l'engin (les animaux à l'intérieur des casiers empêchant ceux de l'extérieur d'entrer). L'utilisation des prises par casier comme indice de l'abondance représente une donnée intéressante pour la gestion des pêches et les études écologiques. Toutefois, les corrélations entre les prises et l'abondance n'ont pas été bien établies, sans doute à cause des nombreux facteurs influant sur le potentiel de capture (par ex. : stade de la mue et cycles de reproduction, sexe, taille de l'animal, cycles lunaires et diurnes, température, mouvement de l'eau). Des méthodes pour effectuer des relevés sur la pêche aux casiers, mesurer le potentiel de capture, et comparer des stratégies de pêche font l'objet d'une analyse critique.

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Traps are among the most versatile and powerful of fishing gears, and are responsible for a high value of landings. They fish unattended, are suitable for most bottom types and a large depth range, they are inexpensive and robust, and requirements for engine power and deck equipment are modest. For some species a very large area of bottom can be fished per day. The species and the size range can be targeted through the choice of trap design and bait, and the catch is usually live. These are desirable qualities for a fishing gear because bycatch is minimal, undersized animals can be returned to the water unharmed, the retained catch is of high quality, and the cost of fishing is low. Based on FAO landing statistics (Anon 1987), I estimate the annual world catch of decapods by traps at 560 000 t with a landed value of U.S. $2.3 billion (assuming $10/kg for lobsters, $2/kg for crabs, and $1/kg for shrimp). Large landings of tropical reef fish, and smaller amounts of freshwater fish and marine molluscs are also taken by traps.

Many factors affect catch rates by traps, e.g. soak time, trap saturation, the physical habitat, trap design, bait, and the life cycle stage of the target species. These subjects have been discussed in one or more reviews by Hancock and Simpson (1962); Bennett and Brown (1979); Elner (1980); and Krouse (1989). This paper includes additional studies and places emphasis on quantitative results and methods of data collection. Catch rates as an index of abundance are also considered. Trapping studies historically appeared most often in the gray literature, but have recently increased in the primary literature. Both sources have included an unfortunate number of studies with weak experimental methodology. The suitability of different methods and suggestions for future research are included. Studies on decapod Crustacea are most thoroughly reviewed, although finfish, gastropods, and amphipods are also included.

Fishing Strategy

Soak time

The objective of many studies on soak time (the elapsed time between setting and hauling) has been to convert catch per trap to an index of abundance, but the results can also be applied to developing fishing strategy. Here, fishing strategy is defined as optimizing fishing effort for greatest net earnings. The relationship between soak time and catch per unit of gear has been studied for at least five decades, initially in relation to finfish catch by nets and longlines.

Two classic studies illustrate the near inevitable decreasing catch per day with increasing soak time. In the first study Hile (quoted in Van Oosten 1935) used catch statistics on total weight of fish caught in impounding nets fished for 1 to 5 d. The catch peaked after 4 d at only 46% higher than the catch after 1 d. In the second, Kennedy (1951) interviewed fishermen at point of landing to collect data on fish catches in gillnets after 1-and 2-d soaks. Whereas the catch from the 2-d soak was larger, the difference decreased from nearly double if the first days catch was small, to only 10% if the first days catch was large. Also, the catch appeared to approach an asymptote characteristic of the gear. Thus, he established that future catch was at least partly dependent on past catch. Using the catch data for 1- and 2-d soaks and a constant maximum as an asymptote, he drew freehand curves to axes of catch/net and days soaked for several fishing areas. The more rapidly the curve approached the asymptote the greater the fish density was presumed to be.

The relationship between catch and soak time was generalized for longlines and gillnets by Gulland (1955) and for gillnets by Beverton and Holt (1957) modeled on Kennedy's curves. Using the symbols of Beverton and Holt

$$[1] \quad C_t = C_\infty((1 - e^{-At}),$$

where C_t is the catch per net or longline after soak time t, C_∞ is the asymptotic catch level, and A is the rate at which the curve approaches the asymptote. This is a von Bertalanffy type equation where the solution for the constants, A and C_∞, are obtained from a regression line fit to successive values of C_t and C_{t+1} (Walford line, c.f. Munro 1974 or Ricker 1975, p. 222). A regression line can also be fit to data which are not collected at equally spaced soak times using an iterative method (e.g. Chasen 1975). Sinoda and Kobayasi (1960) and Miller (1983a) applied equation (1) to trap catches of *Chionoecetes* sp. Somerton and Merritt (1986) added a term for escape (B = probability of a crab escaping per day) of *Chionoecetes* from king crab traps.

$$[2] \quad C_t = \frac{AC_\infty}{B - A}(e^{At} - e^{-Bt})$$

The parameters of the above models have been assigned a variety of biological interpretations. Gulland (1955) interpreted C_∞ as a maximum catch characteristic of a gillnet or longline, and A as an index of fish abundance. Sinoda and Kobayasi (1969) mentioned crab density and bait attractiveness as affecting both C_∞ and A. Somerton and Merritt (1986) considered C_∞ to be a function of crab density and speculated that A might be as well. Whereas C_∞ could be characteristic of a fixed length of gillnet or a given number of hooks on a longline, it is likely not a constant for a particular trap design. Different values have been obtained with identical traps fished by the same investigator at different times or locations (Hughes et al. 1970; Munro 1974; Miller 1978a). It is easy to visualize animal density influencing A because the higher the density the sooner the first animals reach a trap and the steeper the initial slope of the plot of catch versus time. If C_∞ is reached when the bait loses its attractiveness, then C_∞ is a function of density as well as how long the bait remains effective, since it simply measures the number of animals reaching a trap during a time interval. In later sections we see that the threshold of odor concentration necessary to attract an animal can vary with life history stage and appetite, that existing catch can have a repelling effect on new arrivals, and that correlations between catch and density are disappointing. A good test of the dependence of C_∞ or A on density would require their measurement in two or more locations matched in every way except density, and density measured by an independent means.

Another relationship was used by Cleaver (1949), for *Cancer magister*, and by Skud (1979), for *Homarus americanus*. They fit least squares regressions to plots of catch per trap day (C_t/t) against soak time (t) in days.

$$[3] \quad C_t/t = at^{-b}$$

Austin (1977) simply plotted catch per trap (C_t) for *Panulirus argus* against soak time (t) in days and also fit a least squares regression.

$$[4] \quad Q_t = at^{(1-b)}$$

Although equation (4) provided a reasonable fit to several data sets (Miller 1983a), its prediction of a constantly increasing catch with soak is theoretically troublesome.

I see no obvious choice for the best model. If there are sufficient data for a good freehand fit of a curve, this should be as acceptable as any of the above.

Most studies relating catch and soak time are post hoc experiments based on fishermen's records. However, these data have considerable risk of bias. The traps may saturate in less than the shortest soak time (rarely less than 1 d in commercial fisheries) resulting in no change in catch with longer soaks, or a decrease in catch if the captured animals can escape. Data will be incomplete if discards are not recorded or if the fishermen are simply not interested in accurate records. Equally serious, a fisherman's choice of soak time can be dependent on, not independent of, catch rate. For example, a fisherman may use long soaks (1) to increase landings when catch rates are low or, (2) to decrease landings when catch rates are high but weekly landing quotas are being imposed, or (3) to decrease time spent fishing when markets are poor. Bait, trap design, and fishing location also vary among fishermen and affect catch rates. Finally, one fishermen's strategy may be to set few traps and haul them frequently, whereas another's may be to set many traps and haul them infrequently. Thus, soak time is preferably a controlled variable in the experimental design rather than a variable dictated by the exigencies of the fishery.

Studies based on fishermen's records typically show maximum catch per trap haul after long soaks (3–12 d), or no correlation of catch with soak time (Table 1). (In studies where catches increased slowly over long periods, the first time the catch reached >90% of the peak was chosen as the maximum.) In several cases catches decreased after long soak times, indicating more escape than entry. On the other hand, studies where soak times were controlled experimentally typically showed shorter times to maximum catch (Table 2), i.e. 0.2–2 d in six of eight cases where maximum catch was not at the shortest soak time. Exceptions not included in Table 2 were for unbaited Antillean fish traps. Maximum catches occurred after 7–12 d (Munro 1974), about 5 d (Stevenson and Stuart-Sharkey 1980), and >4 d (Miller and Hunte 1987).

Soak time may also affect the sizes of animals caught. *Cancer magister* were fished experimentally for 1, 2, 5, 10, and 20-d soaks (Smith and Jamieson 1989). Catch rates of crabs <135 and ≥135 mm CL were greatest after 1-d and ≥5-d soaks, respectively. Escapement must have been responsible for the declining catch of small crabs, and indeed these sizes could escape through the trap mesh. A large foraging range may have contributed to the catch of large crabs increasing over time. On

TABLE 1. Ratios of catch per trap at the time of first observation and at the time of maximum catch; data from fishermen's records.

Species	Soak time (d)		Approximate catch ratio	Source
	First observation	Maximum catch		
Buccinum undatum	1	1–7	1:1[a]	Hancock (1963, figure 6A)
Paralithodes camtschatica	1	4–10	1:1.9[b]	Rothschild et al. (1970, table 1.4)
Chionoecetes japonicus	1	5	1:1.8	Sinoda and Kobayasi (1969, Figure 4)
Panulirus argus	2	12	1:1.4	Robinson and Dimitriou (1963, figure 5)
Panulirus cygnus	1	1–3	1:1[a]	Morgan (1979)
Homarus gammarus	1	1–5	1:1[a]	Bennett and Lovewell (1977, figure 2)
Homarus americanus	1	3	1:1.3[c]	Thomas (1973, table 8)
	1	5–9	1:2.4-4.3[d]	Skud (1979, table 75)
	1	7	1:1.3[e]	Fogarty and Borden (1980, figure 1)
	1	3	1:1.8	Auster (1986, table 1)
Cancer pagurus, male	1	4	1:1.7[d]	Bennett (1974, figure 3)
female	1	4	1:1.2[d]	(figure 5)
Cancer magister	1	5	1:1.2[f]	Gotshall (1978, table 2)
Geryon maritae	1	1–4	1:1[a]	Melville-Smith (1988, table IV, text)

[a]Either no trend or decreasing catch with soak time.
[b]Mean for 5 yr.
[c]Mean for 3 yr.
[d]Results variable among months.
[e]Legal sized lobsters in traps with escape vents.
[f]Mean of December–March for 4 yr.

TABLE 2. Ratios of catch per trap at the time of first observation and at the time of maximum catch; data from experimental fishing.

Species	Soak time (d)		Approximate catch ratio	Source
	First observation	Maximum catch		
Anoploma fimbria	0.5	1	1:1.3	Hughes et al. (1970)
Mixed tropical fish	1	2	1:1.6	Munro (1974, figure 9)
Cancer productus	0.1	0.2–0.5	1:1.6	Miller (1978a, figures 3,4,7)
Cancer magister	0.2	0.4	1:1.2	Miller (1978a, figure 9)
<135 mm CW	1	1	1:1	Smith and Jamieson (1989, figure 7)
≥135 mm CW	1	5	1:1.4	
Chionoecetes opilio	0.5	1.0	1:1.3	Miller (1981)
Pandalus platyceros				Boutilier and Sloan (1987, figure 2)
Small traps	1	1	1:1	
Large traps	1	4	1:1.6	
Lithodes aequispina	1	2	1:1.8	Sloan and Robinson (1985, table 1)

the other hand, soak time had a negligible effect on sizes of *Chinoecetes opilio* (Miller 1976) and *Pandalus platyceros* (Boutillier and Sloan 1988) caught.

Even though many studies have been published on the relationship of catch and soak time, there is justification for more careful experiments including time as a controlled variable, including short soak times, and including a record of sizes caught. An experiment should be preceded by a uniformity trial (fishing identical traps in an identical manner over the proposed study area) to insure catches are similar throughout the area. Careful attention to standardizing bait quantity and quality, time of day of setting and hauling, trap spacing, state of repair of traps, and randomization of treatments (e.g. soak times) in space and time requires that an investigator do his own fishing rather than obtain data from fishermen (Miller 1983b).

Catch per trap has been standardized for soak time when using trap catches as indices of abundance. Using the relationship between catch per trap and soak time given in equation (1), Sinoda and Kobayasi (1969) calculated the following weighting factor based on rates of entry.

$$W = \frac{1-e^{-A}}{1-e} - At$$

Somerton and Merritt (1986) used a weighting factor including rates of entry and escape based on equation (2).

$$W = \frac{e^{-A}-e^{-B}}{e^{-At}-e^{-Bt}}$$

Multiplying the catch per trap at t days soak by one of these factors gave the catch corrected to a 1-d soak.

$$C_{t=1} = WC_t$$

Rothschild et al. (1970) used a "generalized ANOVA routine" to obtain weighting constants to correct catches to a standard 2-d soak.

Correction for soak time may be unnecessary for commercial catch data. In my experience fishermen use long enough soak times that catches are usually at asymptotic levels. Also, effects of such factors as bait quantity and quality, temperature, molt and reproductive cycle, animal size, and trap density and design (Hancock and Simpson 1962; later sections of this review) can be large by comparison, but unmeasured.

Trapping surveys directed by scientists would best be carried out using a single soak time, but if other important variables are held constant, and catches have not reached asymptotic levels, a correction for soak time may be worthwhile.

Soak time is clearly a component of fishing strategy. A fishery is at its economic optimum when the marginal revenue per unit of effort equals the marginal cost per unit of effort (Anderson 1977). In trap fisheries the unit of effort is the trap haul. A fisherman needs to decide how many traps to own and how often to fish them, e.g. to own 100 traps and fish them all daily, or to own 200 and fish one-half each day. If we assume that the cost of operating the vessel, the number of traps hauled per day, and the number of days fished are fixed, then the cost of owning more traps is the marginal cost and the added catch from longer soaks gives the marginal revenue. The marginal revenue equals marginal cost when

$$P\Delta C - \Delta K = 0.$$

P is price/kg of catch, ΔC is the change in catch per trap haul from increasing soak time (e.g. from 1 to 2 d), and ΔK is the increase in trap cost per haul resulting from increasing the number of traps owned (K = [cost/trap year]/[number of hauls/trap year]). These considerations are discussed further by Austin (1977) and Miller (1983a).

Gear Saturation

A trap's present catch reduces its potential for additional catch. This is called the 'saturation effect.' Munro (1974) made a prodigious number of SCUBA observations on catches of tropical reef fish in unbaited Antillean fish traps. By enumerating the catch by species, color phase, and sex every 2–3 d he obtained minimum estimates of the number of fish entering and escaping between visits. The cumulative entries, i.e. the number present plus the number that escaped, continued to increase linearly throughout the 14-d observation period. The plot of the actual catch diverged from cumulative entry within a few days, became asymptotic by 7–12 d, and was only about 50% of the cumulative entry by 14 d. From these results Munro postulated that the actual catch resulted from a constant number of fish entering and constant proportion escaping daily. Thus, the asymptote increases with rate of entry, and is reached when the rate of entry equals the rate of escape.

In field experiments with baited top entry traps, Miller (1978a) showed that the saturation effect started limiting catches of *Cancer productus* within a few hours, but escape was not the only mechanism operating. Traps were fished in pairs and hauled every 2 h for 12 h. From one trap, the crab catch was counted and the trap carefully returned to the bottom with its catch still inside. From the second trap the catch was counted and removed, and the trap returned to the bottom empty. Bait was renewed in both traps often enough to ensure that it was

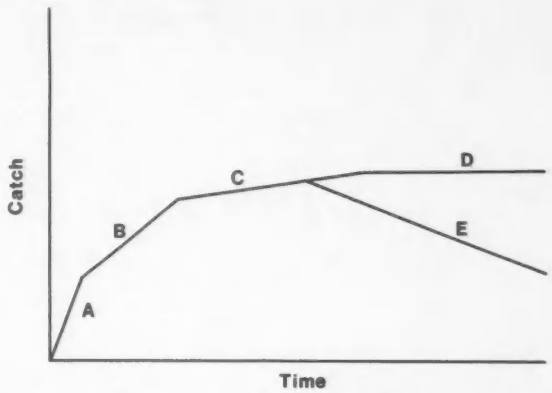

FIG. 1. A generalized relationship between trap-catch and soak-time. Letters identify line segments discussed in the section on gear saturation.

fresh and in excess. Thus, the only difference between the two traps was a greater number of crabs in the trap not emptied. Cumulative catches in the emptied traps were significantly higher than actual catches in unemptied traps by 4 h, and by 12 h the difference was 2.7 times.

Because crabs could escape from traps in the above experiment, second and third experiments tested the hypothesis that the saturation effect was due to escapement. Traps were again fished in pairs, one with a plastic collar which prevented escape entirely, and one without a collar, but neither were emptied. By 12 h the traps with collars caught 60% more crabs than traps without, showing that escapement was a factor. In the next experiment, similar to the first except using only traps with collars, cumulative catches in emptied traps were significantly higher by 8 h and 70% higher by 12 h than in traps not emptied. Thus, both escapement and reduced entry contributed to the saturation effect in the first experiment.

Side entrance traps fitted with one-way doors to prevent escape were fished for *Cancer magister* in a manner similar to the *C. productus* experiments (Miller 1978a). Entry to unemptied traps practically stopped by the first observation at 5 h, whereas emptied traps captured about the same number of crabs as unemptied traps by each observation of 5, 10, 24, and 48 h. This result indicates that the typical 48-h soak time used in the fishery for this species is far longer than necessary. It also indicates that the bait can attract an order of magnitude more crabs than a trap catches, and that potential for improved trap design or fishing strategy is very large.

Possible mechanisms by which entry is reduced have not been tested experimentally, but crabs inside seem to intimidate those outside which would otherwise enter. The intimidation may result from one or a combination of odor, sound, or threatening posture, but odor appears the most likely. Miller (1978b) observed frequent agonistic interactions between crabs inside and outside traps, but saw no evidence that these limited the catch. However, in this and another series of laboratory observations (Miller 1978c) including *Cancer productus*, *C. irroratus*, and *Hyas araneus*, the frequency of approaches to traps decreased as the catches increased, especially after the asymptotic catch levels were reached. This suggests that the intimidation takes place at a distance, as could best be effected by odor. Interestingly, Zimmer-Faust et al. (1984) found that ammonium, an excretory product of decapods, decreased the

feeding response of *Panulirus interruptus* to the attractants glycine and succinic acid. Better understanding of the mechanism(s) of trap saturation could lead to improved fishing strategy or trap design.

Figure 1 shows the relationship between catch by a baited trap and soak time, as generalized from the studies referenced in Tables 1 and 2. The following paragraphs discuss possible behavioral causes of each segment of the curve.

The initial high catch rate (segment A, Fig. 1) is expected because of a high concentration of odor release from newly immersed bait, and because of proximity of the target species to the trap. Mackie et al. (1980) showed that release of amino acids from plaice flesh decreased from about 40 mg/h to about 5 mg/h over the first 6 h, then stabilized at about 3 mg/h for the next 18 h. Crustaceans can accurately track an odor trail. McLeese (1973a) showed that *Homarus americanus* consistently walked upstream to a source of bait odor, and that it followed a dye labeled odor trail laid down in still water with a pipette. The planktonic shrimp, *Acetes sibogae australis*, followed trails of falling food or blotting paper soaked in attractant; both odor sources were labeled with dye (Hamner and Hamner 1977). *Cancer irroratus* also closely followed a dye-labeled odor trail in spite of meandering of the current through a 30° arc (Miller 1980). In both the *Acetes* and *Cancer* studies a dye trail alone illicited no response. In the field, Busdosh et al. (1982) observed amphipods (*Boeckosimus affinis*) responding to bait odor by rapid directed swimming within an arc of <20° for a distance of at least 25 m downstream.

In segment B catch increases at a lower rate, presumably because bait odor is diminishing and the saturation effect is beginning. The bait odor released would decrease with time, and odor concentration would decrease downstream as it became diluted in an ever increasing volume. Both the intensity of response and the fraction of decapods responding to food odor change with odor concentration, as has been shown for *Homarus americanus* (McLeese 1973b), *Cancer antennarius* (Fuzessery and Childress 1975), *Callinectes sapidus* (Pearson and Olla 1977), *Cancer magister* (Pearson et al. 1979), and *Panulirus interruptus* (Zimmer-Faust and Case 1983). *Callinectes sapidus* detected (as indicated by increased rate of antennule flicking) a remarkable low 10^{-15} g/L of freeze-dried clam extract, although feeding response for this and five other decapod species (reviewed in Pearson and Olla 1977) required an odor concentration of 10^{-2} to 10^{-7} g/L.

In segment C the catch does not reach an asymptote but increases slowly. This requires continued attraction of the bait. After the area covered by the odor plume has been 'fished out' additional catch could come from animals wandering into this area or from a shift in current direction which moves the odor plume over a new area (Busdosh et al. 1982). Decreased influence of the saturation effect could also contribute to catch. Aggressive behavior of group-held decapods decreased over time for *Homarus americanus* (Dunham 1972; Zeitlin-Hale and Sastry 1978), *Pagurus bernhardus* (Hazlett 1968), *Orconectes virilis* (Bovbjerg and Stephen 1971), and *Carcinus mediterraneus* (Vannini 1981). Nitrogen production per individual *C. mediterraneus* increased with animal density but decreased slightly over time. However, high animal biomass in a low volume of stagnant water complicated interpretation of the results.

The unchanging catch of segment D is presumed due to loss of bait attraction or, rarely, to the trap becoming full. If escape was possible it could also result from a balance between entry and escape as hypothesized by Munro (1974). The decrease shown by segment E could only occur when escape exceeds entry.

Although the curves in Fig. 1 are typical of *Panulirus* spp. catches (Robinson and Dimitriou 1963; Morgan 1979) the saturation effect may not be as important for these as for many other species. Panulirids are gregarious, they cohabit in burrows, and live conspecifics in traps are effective bait (Lyons and Kennedy 1982).

Temperature

Catchability of decapods often increases with temperature because the activity and appetite of the target species, and the rate of diffusion of the bait molecules increase with temperature (Morrissy 1975).

Branford (1979) measured *Homarus gammarus* locomotor activity and food consumption in the laboratory, and catch per unit effort in the field. Over the range of 7–14°C, food consumption, activity, and catch rates increased by factors of 3, 2, and 2, respectively for males. Female response to temperature was more equivocal.

Homarus americanus walking rate increased by a factor of 2.3 from 2–10°C and catch rate in the field increased by an order of magnitude from 3–11°C (McLeese and Wilder 1958). Paloheimo (1963) used the walking rate–temperature relationship from McLeese and Wilder to adjust catch/trap data from the fishery before estimating population size with the DeLury (1947) method. Ennis et al. (1982) used a similar temperature–walking rate relationship to adjust commercial catch/trap data before calculating population size with the Leslie (1952) method.

Catchability coefficients for *Panulirus cygnus* were positively correlated with temperature in three extensive studies using experimental fishing in Western Australia. From figure 8 in Chittleborough (1970) one can calculate that catchability coefficients increased by a factor of three from 16–22°C. However, Chittleborough noted that unmeasured variables such as natural food availability may have changed concurrently. Morgan's (1974b) data showed a doubling of the catchability coefficient from 18–24°C, although temperature explained only 25% of the variation. By combining all of temperature, salinity, and percentage of the population in premolt condition he was able to account for 49% of the variation in catchability. Crayfish (*Cherax tenuimanus*) were fished with open hoop nets from an experimental pond, then the pond was drained to measure density and other parameters of the population (Morrissy and Caputi 1981). Over a temperature range of 14–22°C catchabilities of large and small crayfish increased by factors of about two and five, respectively. Temperature accounted for 31% of the variability in catch/trap of a portunid crab from eastern Australia (Williams and Hill 1982).

Sex

Females, especially mature females, are often less vulnerable to traps than males. In reference to *Homarus gammarus*, Branford (1979) stated that "Average food consumption in the laboratory is in the ratio of 3 (male):2(unberried female):1 (berried female), and it is therefore probable that average catchability in baited traps will be in the same order." Templeman and Tibbo (1945) confirmed from laboratory observation that egg bearing *H. americanus* also had a small appetite, at least until a few weeks before hatching when food intake increased in spite of little change in temperature. Ropes (1968) noted from dredge

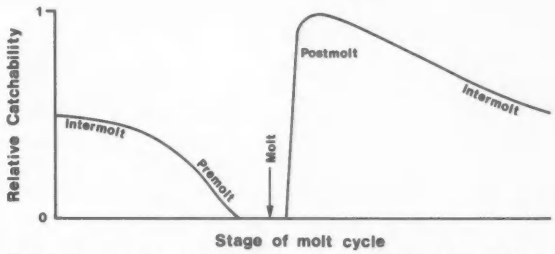

FIG. 2. Generalized relationship between catchability and stage of molt cycle.

collections of *Carcinus maenas* that fewer ovigerous than non-ovigerous females had food in their stomaches.

Ennis (1978; and Department of Fisheries and Oceans, St. Johns, Nfld., pers. comm.) collected *H. americanus* by diving and trapping in the same area at about the same time. Large (81–105 mm CL) males and females were equally abundant (50:51) in diver samples, but males were three times as abundant in trap samples (1039:319). The selectivity for males was less pronounced in smaller lobsters (61–80 mm CL). The diver caught ratio was 0.5:1 (70:153)M:F and the trap caught ratio was 0.8:1 (122:162)M:F.

Morgan (1979) compared sex ratios of juvenile *Panulirus cygnus* from trap-caught and diver-caught samples. The sex ratios from the two capture methods did not differ for smaller animals, but larger females were underrepresented in trap-caught samples (F:M diver-caught 43:57, F:M trap-caught 31:69).

Diver-caught and trap-caught *Jasus lalandii* were 45 and 21% male, respectively, in November–December; by February–March males were a similar 41% of diver catches, but had increased to 56% of trap catches (Pollock and DeB. Beyers 1979). Low catchability of males in November–December was associated with a high incidence of molting, whereas females molted earlier in the year.

Unfortunately, trap catches alone cannot distinguish between catchability and distribution as causes of observed sex ratios. Templeman (1939) illustrated the problem with trap catches for *H. americanus*. Prior to the molting season males greatly predominated at all depths. Immediately after the molting season males predominated at 8–10 m and females at 15–22 m. Howard (1982) noted that ovigerous *Cancer pagurus* were rarely caught by divers or by traps. However, he discovered a brooding area where 77% of the diver-caught but only 2% of trap-caught crabs were ovigerous females. Krouse (1980) observed substantial changes in sex ratios of trap-caught *Cancer irroratus* and *C. borealis* throughout the year. Migration, molting, and mating were all mentioned as possible causes.

Molt cycle

Independent of environmental influences and reproductive cycle, catchability slowly decreases as ecdysis approaches, then drops to near zero for several days before and after ecdysis (Fig. 2). The period of very low catchability totals 10–15% of the molt cycle. As soon as the new shell is hard enough for feeding to begin catchability increases rapidly to a maximum, then gradually decreases to an intermolt level. Because catchability (and catch rate) is highest shortly after the molt, if molting in the population is synchronized, this would be a good time to conduct trapping surveys of animal abundance or to conduct experiments using traps. The following studies support the pattern shown in Fig. 2.

Catchability and molt cycle in *Panulirus cygnus* has shown strong correlation in both field and laboratory studies. Morgan (1974b) observed, by diving, the fraction of *P. cygnus* in premolt condition, then by fishing the surveyed area with traps he determined that these animals were only one-third as vulnerable as those not in premolt condition. Chittleborough (1970) observed a decrease in average catchability during the molting period and a rapid increase immediately afterward. This was based on monthly fishing in an area where density was measured by a mark–recapture. From careful measurements of daily feeding rates in the laboratory, Chittleborough (1975) found that feeding began within 2 d after molting, peaked 5 d after molting and gradually returned to near zero about 9 d before the next molt in a molt cycle of about 50 d. Also in the laboratory, Morgan (1978) determined that locomotor activity was greater for intermolt than for premolt animals at each of four temperatures.

Studies on several other species also support the correlation between molt and catchability. The daily food intake of *Panulirus argus* was less than one-half the mean of the entire 65-d molt cycle during 7 d premolt and 2 d postmolt (Lipcius and Herrnkind 1982). However, daily rates of locomotor and foraging activity were in only moderate agreement with daily food intake. In an annual molt cycle, *Jasus lalandii* did not feed for 44 d pre- and 34 d postmolt (Zoutendyk 1988). Catchability of *Cherax tenuimanus* fished with hoop nets in an experimental pond, peaked at early intermolt and dropped to zero immediately before and after ecdysis (Morissy and Caputi 1981). Catch per trap of a portunid crab was negatively correlated ($r = -0.43$) with percentage of recently molted crabs in the catch (Williams and Hill 1982). In combination with temperature the multiple correlation improved to 81%. Based on field collections, all *Homarus americanus* in postmolt had stomaches at least one-half full, those in premolt no more than one-quarter full, and those in intermolt ranged from empty to full (Weiss 1970). In the laboratory *Chionoecetes opilio* did not feed for 2–6 wk before molt and 3–4 wk after molt (O'Halloran and O'Dor 1988). Hancock (1965) associated reduced catch–effort with molting in the fishery for *Cancer pagurus*, as did Templeman (1939) for *Homarus americanus*, and Newman and Pollock (1974) and Pollock and DeB. Beyers (1979) for *Jasus lalandei*. In the last two studies molting periods were determined from diver-collected samples.

Diurnal and Lunar Cycles

Activity in decapods ususlly peaks near dawn and dusk, or is simply higher during night than day. Chapman et al. (1975) studied *Nephrops norvegicus* in the field using photography, television, and acoustic tags. Activity was greatest just before sunrise and after sunset, but peaked about 2 h later in the morning and 2 h earlier in the evening at 93 m than at 42 m, corresponding to the times of similar light intensity. This and other decapod species observed to be most active at twilight or night are listed in Table 3. Trap catches of *Chionoecetes opilio* did not differ for day and night, probably because the diurnal light variation at 200 m was insufficient to cue changes in activity (Miller 1983a).

Catches per trap of *Panulirus cygnus* (Chittleborough 1970; Morgan 1974b) and *Panulirus argus* (Sutcliffe 1956) were high at new moon and low at full moon. In the laboratory nocturnal

TABLE 3. Decapods for which peaks in activity have been observed at night or twilight.

Species	Study location, observation	Time of peak activity	Source
Carcinus meanas	Field, feeding	Night	Kitching and Ebling (1967)
	Laboratory, movement	Night	Naylor (1960)
Cherax tenuimanus	Field, trapping	Night	Morrissy (1975)
Homarus americanus	Laboratory, shelter occupancy	Night	Cobb (1971)
	Laboratory, feeding and movement	Night	Lawton (1987)
Homarus gammarus	Laboratory, movement	Night	Branford (1979)
Jasus lalandii	Laboratory, feeding	Dusk	Fielder (1965a)
Leander serratus	Field, feeding	Night	Kitching and Ebling (1967)
Nephrops norvegicus	Field, number out of shelter	Dusk and dawn	Chapman et al. (1975); Chapman and Howard (1979)
Pandalus platyceros	Field, movement	Night	Boutillier (1985)
Panulirus cygnus	Laboratory, movement	Dusk	Morgan (1978)
	Field, shelter occupancy	Night	Cobb (1981)
	Field, movement	Night	Jernakoff (1987)
Panulirus argus	Laboratory, movement	Dusk and dawn	Casterlin and Reynolds (1979)
	Laboratory, movement	Dusk	Lipcius and Herrnkind (1982)
Panulirus japonicus	Field, trapping	Dusk and dawn	Kubo and Ishiwata (1964)
	Laboratory, feeding	Dusk to 0200	Kubo and Masuda (1964)
Penaeus duorarum	Laboratory, movement	Dusk	Hughes (1968)
Portunus puber	Field, feeding	Midnight to dawn	Kitching and Ebling (1967)

illumination simulating moonlight reduced activity of juvenile *P. cygnus* by about 35% (Hindley 1976) and reduced activity of *P. argus* by a similar amount (Sutcliffe 1956).

Water motion

Near-bottom current speeds greater than about 27 cm/s effectively eliminated movement of European lobster (*Homarus gammarus*) in flume tests (Howard and Nunny 1983). The critical velocity for wave induced water motion would be even lower because reversal of direction every several seconds would reduce the benefit of posturing to minimize current drag. The authors concluded that water motion would affect lobster behavior, including response to bait odor, much of the time in most coastal areas of the British Isles. However, they do acknowledge that field environments which are more rugose than the laboratory test environment would provide shelter from currents.

Time lapse photography of bait in the field revealed little change in foraging activity of *Cancer pagurus* through a tidal cycle. Off bottom current speed ranged from 5–80 cm/s (Howard 1980).

Size

Behavioral mechanisms and trap design can affect size of decapods in trap catches. A trap entrance can be too small for big animals to enter or openings in the material covering traps can allow small animals to escape. Large animals are expected to have larger foraging ranges than small animals because of greater food requirements and faster walking rates (Morrissy 1973). Also, more time per day was spent foraging by large than small *Nephrops* (Chapman and Howard 1979) and *Homarus americanus* (Lawton 1987).

By means of aggressive encounters large animals may deter small animals from entering traps. These encounters occur outside traps while animals are searching for the entrance, and between animals inside and outside traps (Miller 1980; Bjordal 1986), although encounters outside traps probably influence catch the most. Laboratory behavior studies on several species of Decapoda have demonstrated that larger conspecifics usually win agonistic encounters: e.g. *Pagurus longicarpus* (Allee and Douglas 1945), *Procambarus alleni* (Bovbjerg 1956), *Jasus lalandii* (Fielder 1965b), *Mictyris longicarpus* (Cameron 1966), *Heloecius cordiformis* (Griffin 1968), *Homarus americanus* (Schrivener 1971), and *Menippe mercenaria* (Sinclair 1977). The larger the size difference the more likely the win (Bovbjerg 1956; Schrivener 1971), until at very different sizes conspecifics appear to ignore one another (Fielder 1965b; Schrivener 1971).

The literature includes several examples of smaller individuals of decapod species being underrepresented in trap catches (Table 4). Smith (1944) tagged trap-caught American lobsters before the fishing season in five locations, then recorded the sizes of tagged animals recaptured by the fishery. A higher fraction of large lobsters were recaptured at all locations. Carroll (1982, TERA Corporation, Avila Beach, California, pers. comm.) and Williams and Hill (1982) used a similar method for a *Cancer* and a portunid crab, respectively except that the authors did both the initial and final trapping instead of relying on a fishery. Catchability was about four-fold higher for larger animals. As noted by Williams and Hill, capturing and recapturing by trapping included only animals initially vulnerable to trapping. If one of the captures had been by a different method the bias for large animals may have been even larger. Ennis (1978; and pers. comm.) obtained size frequencies of American lobster by diving ($N = 278$) and trapping ($N = 1380$) in the same area at about the same time. Assuming the diver samples were an unbiased index of abundance, traps were highly selective for larger lobsters. Miller (1989) also used diving and trapping for American lobster and a *Cancer* crab with similar high trap selectivity. Morrissy and Caputi (1981) found that catchability of crayfish in hoopnets increased by three to seven times over a three-fold increase in carapace length. Pollock and DeB. Beyers (1979) sampled an unusually large number of *Jasus lal-*

andii by diving ($N = 5565$) and trapping ($N = 4074$) during two years and found both larger males and females slightly more trappable.

In contrast to the above results, two studies found that large spiny lobsters have reduced vulnerability to trapping. By fishing traps in a large tank Morgan (1979) showed that male *Panulirus cygnus* 76–85 mm CL were twice as likely, and females of the same size three times as likely to be captured as those >85 mm CL. Newman and Pollock (1974) found male *Jasus lalandii* >100 mm CL present in diver samples at each of five stations, but virtually absent from trap catches. However, as noted above, Pollock and DeB. Beyers (1979) found large males slightly more vulnerable to traps.

Among large berried female *Homarus americanus*, Campbell (1990) found no size selectivity by traps. The range from both diving ($N = 121$) and trapping ($N = 388$) was about 100–160 mm CL and the mean size was 131 mm CL for both groups. These lobsters were larger than those in studies quoted in Table 4.

A logistic curve (Fig. 3) generalizes the relationship between animal size and catchability. Catchability is near zero for a range of small sizes, increases first gradually then rapidly over intermediate sizes, then levels off or possibly even decreases for large sizes. As noted previously details of this relationship can change with sex and soak time.

Interspecific Interactions

Natural predators of target species in traps would logically be expected to reduce catches, but supporting data are scarce. Ritchie (1972) found that 20% of traps set for *Jasus edwardsii* were entered by octopus. Although octopus did not eat all lobsters in traps, the uneaten lobsters were so traumatized that they were dead or nearly so and of no commercial value. Chittleborough (1974) found less than one-half as many *Panulirus cygnus* in traps with octupus as in traps without.

In field experiments Richards et al. (1982) stocked baited American lobster traps with zero, three, eight *Cancer irroratus*, *Cancer borealis*, or *Homarus americanus*. Stocking traps with *H. americanus* reduced the catch of both *Cancer* spp. But, stocking either *Cancer* sp. had no significant effect on the catch of the other, or on *H. americanus* (Table 5). Thus, *H. americanus*, a known predator on both *Cancer* spp., deterred entry of both, but the stocking of *Cancer* spp. did not improve catches of *H. americanus*. The effect of stocking conspecifics is discussed in the next section.

A host of scavenger species can reduce the effectiveness of a trap by eating the bait. I have experienced this with amphipods, crabs, sea urchins, seastars, finfish, and seals.

Natural bait

Dead decapods in traps can greatly reduce catches of conspecifics. Catches with normal bait were compared with catches with normal bait plus dead decapods, and to catches with dead decapods only (Table 6).

Hancock (1974) pointed out that this phenomenon could be used to reduce the catch of nuisance decapod species. Nuisance species compete with the target species for bait and space in the trap and may even be a natural predator. Hancock reduced the catch of *Carcinus maenas* and increased the catch of whelks by adding dead *Carcinus* to the trap. *Carcinus* is a whelk predator. Because dead decapods are so effective in repelling live conspecifics, fishermen must be careful not to leave pieces of the target species in their traps (Hancock 1974).

Whereas dead decapods repel conspecifics, live conspecifics are used as bait in a few fisheries. In the southeastern United States fishery for *Callinectes sapidus*, traps are baited with adult males to attract females about to molt and mate. In a field test including several dates and locations, traps baited with males caught 1.5 times as many premolt females as unbaited traps (Bishop et al. 1984). In the laboratory premolt females responded with courtship display to a combination of adult male odor and sight (Teytaud 1971). The Florida fishery for *Panulirus argus* takes advantage of panulirids' gregarious nature by using undersized lobsters as bait. Traps baited with cowhide plus three, one, or zero undersized lobsters had average catches of 4.2, 1.9, and 1.2, respectively (Lyons and Kennedy 1982).

TABLE 4. Examples of higher catchability for larger decapods.

Species	Capture method	Sizes compared (mm)	Catch ratio	Source
Homarus americanus	Trap-tag-release-trap	60 and 80 CL	1:1.7[a]	Smith (1944)
	Diving-trapping	M 61-80 and 81-105 CL	1:12	Ennis (1978 and
		F 61-80 and 81–105 CL	1:6	pers. comm.)
	Diving-trapping	60–69 and 70–89 CL	1:8[b]	Miller (1989)
Cancer antennarius	Trap-tag-release-trap	85–94 and 95–114 & 125–144 CW	1:3:4	Carroll (1982 and pers. comm.)
Cancer irroratus	Diving-trapping	60–79 and >79 CW	1:5[b]	Miler (1989)
Scylla serrata[c]	Trap-tag-release-trap	M 100 and 150 CW	1:3.3	Williams and Hill
		F 100 and 150 CW	1:4.5	(1982)
Cherax tenuimanus	Trap-population count	1 and 3[d]	1:3–7[e]	Morrissy and Caputi (1981)
Jasus lalandii	Diving-trapping	M 70–80 and >80 CL	1:1.1[f]	Pollock and
		F 70–80 and >80 CL	1:1.1[f]	DeB. Beyers (1979)

[a]Mean of five locations.
[b]Mean of two locations.
[c]Portunid crab.
[d]Relative change in carapace length.
[e]Range at three temperatures.
[f]Mean of 2 yr.

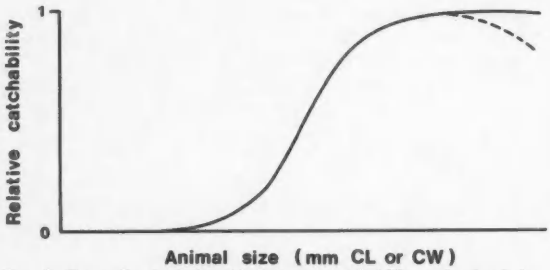

FIG. 3. Generalized relationship between catchability and animal size. Catchability may not change or may decrease slightly for large sizes.

TABLE 5. Mean catch per trap of *Cancer irroratus*, *Cancer borealis*, and *Homarus americanus* in traps prestocked with zero, three or eight individuals of each species (from Richards et al. 1982).

	Species and number stocked								
	C. irroratus			*C. borealis*			*H. americanus*		
Species caught	0	3	8	0	3	8	0	3	8
C. irroratus	9.5	10.1	9.9	8.8	10.9	8.8	7.6	2.4	1.0
C. borealis	1.8	2.8	1.9	1.8	2.9	2.3	1.7	0.9	0.4
H. americanus	0.8	0.8	0.8	0.7	0.6	1.0	1.3	0.7	0.5

This baiting practice is expensive however. Between 10 and 60% as many undersized lobsters are killed by air exposure, starvation, and injuries as legal sized lobsters are taken by the fishery (Hunt et al. 1986). The mechanism for attraction may be olfactory. Zimmer–Faust et al. (1985) discovered in tank experiments that water occupied by either male or female *Panulirus interruptus* attracted both sexes. In the experiments by Richards et al. (1982), stocking American lobster traps with *H. americanus* reduced the catch of conspecifics, whereas stocking with *C. borealis* or *C. irroratus* had no effect on the catch of conspecifics (Table 5).

Bait choice experiments have indicated that fresh bait is chosen over stale bait, marine species are chosen over mammals, and cut bait is chosen over live prey. In the field, fresh flatfish caught more *Homarus gammarus* and *Cancer pagurus* than salted flatfish (Thomas 1953; Edwards and Meaney 1968), and fresh menhaden (*Brevoortia tyrannus*) caught more *Callinectes sapidus* than salted menhaden (Cronin 1950). In the laboratory *Jasus lalandii* chose fresh fish over "stale fish," chose fish and shark over rabbit and horseflesh, but showed no preference between fish and shark or squid and shark (Fielder 1965a). *Homarus gammarus* showed no preference between shore crab and plaice, mackerel and plaice, and squid and plaice when fished with traps in a large tank (Mackie et al. 1980). In the field, live mussels and abalone caught no more *Panulirus interruptus* than unbaited traps, whereas injured mussels and abalone were more effective (Zimmer-Faust and Case 1982). In another series of tests on this species, abalone muscle was a superior bait to tissue from each of sea urchin, shrimp, shark, or mackerel, and to no bait (Zimmer-Faust and Case 1982). Both quantity and quality of molecules diffusing from bait are known to affect decapod response (McLeese 1973b; Zimmer-Faust et al. 1984).

Bait quantity often affects catch rates, but few generalizations are apparent. Traps baited with 46–372 g of abalone muscle had similar catch rates of *Panulirus interruptus*, whereas baits of 18, 7, and 3 g yielded progressively lower catch rates (Zimmer-Faust and Case 1983). In another experiment by the same authors (Zimmer-Faust and Case 1982), weights of abalone in the ratio of 1:2:6:10 provided the same catches of *P. interruptus*, and weights of angel shark in the same ratios gave similar catch rates of crabs (*Cancer antennarius* and *Loxorhyncus grandis*). Increasing squid bait from 1 to 3 kg/trap increased catches of *Chionoecetes opilio* about 50% after both 1-d and 4-d soak times (Miller 1983a). Catches of *Homarus*

TABLE 6. Crab and lobster catches in traps baited with and without dead conspecifics.

Target species	Bait	Catch/trap	Source
Carcinus maenas	Salted skate	3.6	Hancock (1974)
	Salted skate + dead crab	1.4	
	Dead crab	0.7	
Panulirus cygnus	Cattle hock + fish head	10.2	Hancock (1974)
	Cattle hock + fish head +dead lobster	0.6	
Panulirus cygnus	Cattle hock + fish head	12.9	Morgan (1974b)
	Cattle hock + fish head +dead lobster	5.2	
Chionoecetes opilio	Squid	31.0	Miller (1977)
	Squid + dead crab	7.6	
	Dead crab	0.6	
	No bait	0.3	
Cancer pagurus	Fish	5.5	Chapman and
	Fish + dead crab	2.5	Smith (1978)
	Dead crab	0.8	
Paralithodes camtschatica	Fish	11.8	High and
	Dead crab	0.7	Worlund (1979)
	No bait	1.0	
Libinia dubia or *L. emarginata*	Fish	23.5	Richards and
	Fish + dead crab	8.0	Cobb (1987)
Panulirus interruptus	Dead lobster	1[a]	Zimmer-Faust et al.
	No bait	3[a]	(1985)

[a]Ratio of total catches in tank experiments; number of observations not given.

gammarus did not differ when baited with one-half or one and one-half mackerel (Thomas 1954). Three live, undersized *Panulirus argus* were about twice as effective as one *P. argus* in attracting conspecifics (Lyons and Kenney 1982). Over a range of bait sizes of 1–700 g, catches of amphipods increased from 500–70 000 per trap (Sainte-Marie 1986). Unbaited traps captured relatively few *Homarus americanus* (Sheldon and Dow 1975; Pecci et al. 1978), *Homarus gammarus*, *Cancer pagurus* (Mackie et al. 1980). *Chionoecetes opilio* (Miller 1977), *Panulirus interruptus* (Zimmer-Faust and Case 1983), *Paralithodes camtschatica* (High and Worlund 1979), and amphipods (Sainte-Marie 1986).

Artificial bait

The development of an inexpensive artificial bait with good keeping qualities has been attempted many times (c.f. Koyama et al. 1971; Jaeger 1972; Ache et al. 1978). A partial review of the extensive literature on this subject suggests the following generalizations: the best artificial bait is no better attractant than a good natural bait; single compounds are not as attractive as mixtures; and the timing of release of the attractant by the carrier substance is important to bait performance. Fuzzessery and Childress (1975), McLeese (1970), and Shelton and Mackie (1971) demonstrated in the laboratory that mixtures of compounds were more stimulating than single compounds to *Pagurus hirsutiusculus*, *Homarus americanus*, and *Carcinus maenas*, respectively. No mixture was more stimulating than natural bait. Squid muscle extract and a chemical mixture with *L*-amino acids, each contained in a carrier of gypsum (plaster of Paris), gave catches of *Homarus gammarus* similar to those obtained with plaice flesh, the standard bait in the fishery (Mackie et al. 1980). However, the same chemical mixture in agar gave catches only 20% as high as from plaice flesh. Amino acids were continually released from plaice flesh and gypsum for 24 h, whereas none were released from agar after 6 h. Although not tested in traps, gelatin also continually released amino acids over 24 h. In another series by Mackie et al. (1980), an extract of sprat in gypsum and the chemical mixture in gypsum were both as effective as salted mackerel, but only one-half as effective as plaice flesh. Ache et al. (1978) must have used different formulations of carriers, because they found that agar continually released dissolved substances over 3 d, but gelatin dissolved within a few hours.

Response to varying concentrations of bait extracts in the laboratory varied with the type of behavioral response observed and the attractant, but generally increased moderately with order of magnitude increases in concentration. For example, the number of *Homarus americanus* responding increased from 11 to 59% with a 100-fold increase in concentration of cod muscle extract (McLeese 1973b), or 30 to 70% with a 100-fold increase in concentration of herring extract (Daniel and Bayer 1987). *Panulirus argus* response increased three-fold to a 100-fold increase in shrimp extract (Ache et al. 1978).

Effective fishing area

Catch per unit effort (c/f) is related to stock size (N) as

$$c/f = qN$$

where q is the catchability coefficient, defined as the fraction of the stock captured by a unit of fishing effort (Ricker 1975). If stock density (D) is substituted for stock size, and c/f is catch per trap haul, the catchability coefficient becomes

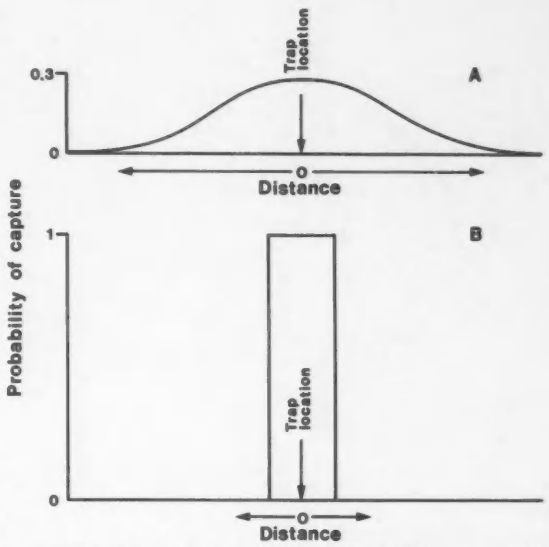

FIG. 4. Probability of capture at different distances from a trap. (A) Area of attraction; (B) effective fishing area or 'q'.

$$q = \frac{c/f}{D}$$

with units expressed in area per trap haul (Chittleborough 1970; Mogan 1974b). In this application, q has also been termed the effective fishing area (Miller 1975; Melville-Smith 1986; Himmelman 1988). If q can be estimated with enough accuracy and precision it can be very useful for converting catch per trap to stock density.

Note however, that q is smaller than the actual area from which the target animals are attracted to a trap. For these areas to be the same, 100% of the target animals must be captured from inside and none from outside the area. Almost certainly, a decreasing fraction would be captured at increasing distance. The effective fishing area is somewhat analogous to the planktologist's concept of water volume cleared by filter feeders. The actual efficiency of removing prey from the water is not known, but the clearance rate is expressed as the equivalent volume from which 100% are removed. A trap's 'area of attraction' is illustrated by the 'fried egg' probability distribution in Fig. 4A, and the equivalent effective fishing area with 100% probability of capture in Fig. 4B. The concept of decreasing probability of capture with increasing distance is supported by the decreasing percentage of decapods responding to reduced bait odor concentration, as reviewed in the previous section. It is also supported by studies which have measured distance and direction moved to a trap.

McQuinn et al. (1988) and Himmelman (1988) reported on different aspects of a study in which 20 to 50 tagged whelks (*Buccinum undatum*) were released at 2, 5, 10, 18, 30, and 50 m on each of four transects radiating from a baited trap. The trap was emptied and rebaited by divers daily for 13–15 d. The experiment was repeated eight times with one trap each time. The area of attraction included within the 2% isopleth of capture probability averaged about 10 times the effective area fished after 1 d (McQuinn et al. 1988). In all eight trials at least one whelk was recaptured from 50 m by the 6th day of fishing

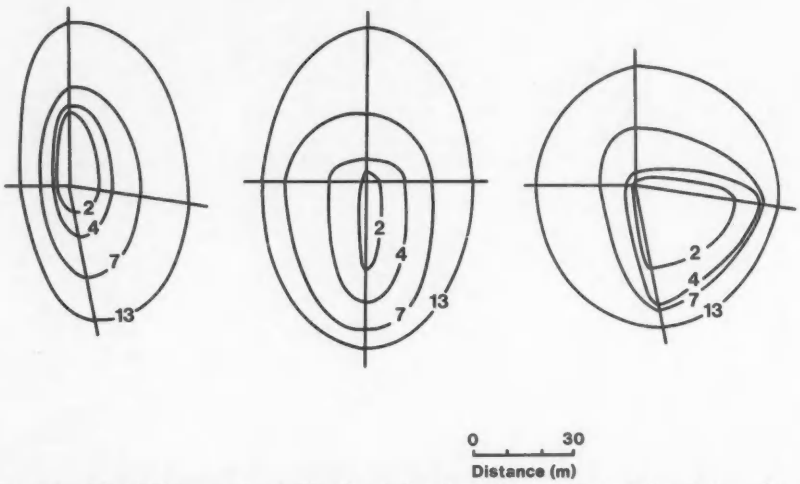

FIG. 5. Isopleths of 25% capture probability for whelks after 2, 4, 7, and 13 d soak time (redrawn from Himmelman 1988).

(Himmelman 1988). Clearly, the area of attraction was far larger than q. Himmelman also provided interesting data on the shape of the area of attraction. Two days after the releases the isopleths of 25% capture probability were typically elliptical and longest on one or two of the four transects. However, as time progressed the capture area became more rounded and more centered on the trap (Fig. 5). Whelks located within the bait odor plume when the trap was set were thought to provide the early captures, whereas whelks which wandered into the plume area were throught to provide the later captures.

Jernakoff and Phillips (1988) measured distance and direction of attraction of spiny lobsters (*Panulirus cygnus*) fitted with electromagnetic tags. Within a 90° angle and 120 m down current from a baited trap, 13 lobsters went to the trap and 20 did not. For all other directions 8 of 50 lobsters went to the trap. The average straight line distance traveled to the trap from down current was 75 m and the maximum distance was the boundary of the tag detection area, 120 m.

Two less successful studies used designs similar to McQuinn et al. (1988) and Himmelman (1988). Marked snails (*Neptunea arthritica*) were released on four axes from a single trap (Ito et al. 1981). However, most recoveries were from only two release points 10 m from the trap, and low recoveries (7%) from the single trial do not warrant interpretation of the area of attraction. Tagged crabs (*Chionoecetes opilio*) were released on a single axis from each of 22 traps (Brethes et al. 1985). Although total releases were large, very low recoveries (1.7%) and questionable assumptions shed doubt on calculations of distance traveled and area fished (Miller 1985).

Elegant mathematical models have predicted that deepsea scavenging amphipods can travel thousands of metres to a bait (e.g. Sainte-Marie and Hargrave 1987). However, since these studies have been conducted in such hostile environments important assumptions were untestable. The response of shallow water amphipods to bait at an Arctic site was observed by divers (Busdosh et al. 1982). The amphipods responded by directed swimming from a distance of at least 30 m downstream of the bait and within an angle of $<20°$.

Estimates of q have been obtained by measuring animal density and catch per trap in approximately the same area and the same time. The two most detailed studies were conducted on *Panulirus cygnus* (>30 and >50 mm CL) in shallow reef habitat in Western Australia. Chittleborough (1970) measured abundance by mark–recapture and measured catchability for five successive Januaries at one to three locations each year. Among locations within years q's were in close agreement (e.g. 144, 164, 174 m^2), but varied from 25–174 m^2 among years. On a reef further north Morgan (1974b) estimated q for 34 of 38 successive months, and also estimated density by mark–recapture. His results for four successive Decembers were 143, 130, 79, and 145 m^2, although the overall range was much larger at 38–196 m^2. Catchability coefficients for *Chionoecetes opilio* (>90 mm CW) in four deepwater (160–245 m) mud habitats in eastern Canada were 2470, 4190, 4470, and 5290 m^2 (Miller 1975); density was measured by bottom photography. Melville-Smith (1986) also used photography to measure density of a deepwater population of *Geryon maritae* (>60 mm CW) on mud bottom, and estimated q at 2160 m^2. Five fish taxa (>100 or >200 mm fork length) were censused by divers on two shallow reefs in Barbados, West Indies (Miller and Hunte 1987). Catchabilities (in unbaited traps) for squirrelfish, angelfish, and a grouper were 335–348 m^2, and for parrotfish and surgeonfish were 134–194 m^2. In eastern Canada *Homarus americanus* and *Cancer irroratus* were censused by divers in shallow rocky habitats with and without kelp cover (Miller 1989). Although catchabilities for the two habitats were in reasonable agreement, catchabilities increased greatly with animal size, e.g. 49 to 373 m^2 for *H. americanus* 60–69 and 70–89 mm CL, respectively and 60 to 276 m^2 for *C. irroratus* 60–79 and >79 mm CW, respectively. Catchability of *Buccinum undatum* (>60 mm shell length) on a shallow sand or mud bottom was 276 m^2 after a 1-d soak (McQuinn et al. 1988). This was for their trial no. 8 in which the whelks were not stressed during tagging. Unlike the above examples, q for *Buccinum* was measured as the product of the area of attraction and probability of capture within the area.

Catchability coefficients have also been calculated from observed catches in traps set at a range of distances such that traps compete for catch to a varying degree. This experiment was conducted by Eggers et al. (1982) fishing for sculpin, and

Gros and Santareli (1986) fishing for whelk. However, required assumptions that the fishing area is centered on the trap and is a specified shape (a circle and ellipse in these studies) could introduce significant bias. We saw above that the fishing area of a whelk trap could assume a variety of shapes and that the area was usually not centered on the trap (Himmelman 1988). The method also assumes that the area of attraction equals q, whereas we have seen that the area of attraction is much larger than q. Adjacent traps could compete for catch even though the q's do not overlap, i.e. probability distributions of the type shown in Fig. 4A could overlap, whereas the type shown in 4B would not. However, catch rates at different trap spacings would be useful for determining optimum spacings for traps in a commercial fishery. Sinoda and Kabayasi (1969) fished crab (*Chionoecetes japonicus*) traps at different spacings and found that catch increased from 33 to 50 m spacing, but remained unchanged at 67 m. Williams and Hill (1982) observed that catches of portunid crabs increased with trap spacings of 50 to 100 m, but did not change at 200 m.

In addition to its use for converting catch per trap to stock density, q is a measure of the fishing power of traps. The following text table compares the fishing power per day of inshore (working 12 h d) and offshore (working 24 h d) snow crab vessels and groundfish trawlers. (The catchability coefficient is from Miller (1975) and the other estimates are based on pers. comm.)

	Crab boats	Trawlers
Inshore	[a]$1.2 \times 10^6 m^2$	[c]$0.5 \times 10^6 m^2$
Offshore	[b]$4.1 \times 10^6 m^2$	[d]$2.9 \times 10^6 m^2$

[a]300 traps/d × 4100 m^2 trap

[b]1000 traps/d × 4100 m^2/trap

[c]12 m trawl width × 5500 m/h tow speed × 8h/d bottom time

[d]30 m trawl width × 6000 m/h tow speed × 16h/d bottom time

This comparison shows that crab boats fish more bottom area than trawlers. A comparison of operating costs would also favor the crab boats.

In conclusion, decapod catchability coefficients measured on shallow bottoms with high relief have been in the tens and hundreds of square metres, whereas values from deep flat habitats have been in thousands of square metres. The larger area for *Chionoecetes* and *Geryon* may reflect the more uniform substrate, lack of home range, or a scarcer food supply. The low q for the whelk on flat bottom is presumably a result of its relatively slow crawling rate. Catchability coefficients based on catch rates at a range of trap spacings are subject to bias. But, experiments on trap spacing have direct application of optimizing trap distances in commercial fishing.

Catch Per Trap as an Index of Abundance

This review began with reasons why traps are good fishing gear. Traps are convenient for measuring animal abundance for the same reasons. Furthermore, alternative sampling gears have serious limitations. Towed nets or dredges foul on rough bottom, damage the catch, catch unwanted animals and debris, and require specialized vessel equipment. Remote photography or television are unsuitable for searching under cover or in turbid water, the target animals are not captured, and the area surveyed is small and difficult to measure accurately. Manned submersibles share the disadvantages of film and video with the addition of high cost. SCUBA is limited to a practical survey depth of 20 m, the search area is small, areas of high relief can be searched only slowly if at all, and specially trained personnel are required.

The bad news about traps is that the catchability of the target species changes with so many biological and physical variables, as discussed in the previous section. A later section discusses additional selectivity associated with trap design. This section reviews studies comparing trap catches to animal density, and considers analytical methods for estimating abundance from catch rates.

Catch Rate Versus Abundance

Morgan (1974a) measured density of *Panulirus cygnus* by mark–recapture methods, using trapping to capture and recapture, in 34 of 38 successive months. On four occasions the proportion of marked lobsters in the population was also surveyed by diving. He found that lobsters which had been caught in traps, marked, and released were more vulnerable to recapture than unmarked lobsters, and used this result to correct differences in trapability. Without the correction, multiple and single mark–recapture methods underestimated the population density by averages of 37 and 44%, respectively. With the correction population densities and catch per trap were highly correlated ($R^2 = 0.74$, calculated from Morgan 1974b), but were not independent since both were based on the same trap catches.

Correlation of *Chionoecetes opilio* density and catch per trap was not significant ($R^2 = 0.1$, $N = 4$), although the variance would have had to be low to show a correlation over the small ranges of 62–142 crabs/ha and 26–43 crabs/trap (Miller 1975). Density was based on bottom photography.

Morrissy and Caputi (1981) generated a multiple regression equation to predict the number of crayfish (*Cherax tenuimanus*) of a given size in a 0.01-ha experimental pond. Under carefully replicated conditions, fast fishing hoop nets were fished 25 times per night on two successive nights on 14 occasions. The true population was counted by draining the pond after each sampling. Temperature, year, and animal size were more important in predicting crayfish number than were sex, molt stage, or first versus second night of fishing. A regression equation including the first three of the above variables was next used to predict population densities from trap catches in 12, 0.1-ha farm ponds. The true crayfish densities, measured by seining the ponds, was only slightly correlated with predicted densities ($R^2 = 0.29$), and that was after excluding data from two ponds because of abnormal environmental conditions. To improve the correlation, the regression equation was further modified by omitting the variable for year and adding variables for turbidity and a coefficient of variation for crayfish weight. Although R^2 was substantially increased to 0.83, the resulting equation is complex, it required many samples to generate, and its predictive value for populations not used in its generation is unknown.

Divers surveyed densities of a tropical reef fish (*Halocentrus ascensionis*) in 30 × 30 m areas were highly correlated with catches in unbaited traps ($r = 0.89$, $N = 19$) (Miller and Hunte 1987). However, density and catches were not correlated for four other taxa.

Homarus americanus and *Cancer irroratus* were trapped and their density measured by diving in adjacent habitats with and

without cover of large kelps (Miller 1989). Catchabilities for both species were in reasonable agreement between habitats within each size group, e.g. 13 versus 14, 51 versus 70, and 213 versus 339 m^2 (Table 4).

Analytical methods

Fishing success methods (Leslie 1952; DeLury 1947) of determining population size for decapods deserve detailed consideration because of their popularity (Paloheimo 1963; Gotshall 1978; Methot and Botsford 1982; Otto 1986; Ennis et al. 1986; Bailey and Elner 1989). The data requirements are modest, the calculations are easy to carry out, and the results are in demand by ecologists and fishery managers. For Leslie (1952), catch per trap (or any measure of catch per unit effort) is plotted on the Y axis against cumulative catch on the X axis; for DeLury (1947), $\log_e$ catch per trap is plotted against cumulative number of trap hauls. The size of the population before fishing began is easily calculated with either method using the parameters from a least squares regression (Ricker 1975). The methods can be used with data collected either from experimental fishing over a short time or from a commercial fishery over a longer time. Their weaknesses originate in their requirements for a time series of catch rates and for the capture of a large portion of the population. The assumptions are the same for both Leslie and DeLury methods and are considered below.

(1) Catchability of the fished population is constant among individuals and is constant over the time of fishing. This assumption would rarely, if ever, be met for decapods. Catchability varies greatly among individuals (e.g. size and sex) and over time (e.g. stage of the molt and reproductive cycles, temperature), as already discussed. Morgan (1974a) trapped *Panulirus cygnus* for 6 d in each of 34 mo. The DeLury method underestimated density by an average of 75%, compared with tag–recapture estimates corrected for unequal catchability. Morrissy (1975) fished for crayfish with hoop nets set 10 times in one night in a small pond. After fishing, the population was censused by draining the pond. The Leslie method underestimated crayfish numbers by 61, 47, and 53% in December, February, and April, respectively. In both studies the large underestimates were attributed to low catchability of some components of the population. That is, the catch per trap decreased faster than population size because the most vulnerable animals were caught first. In both studies replicate trapping was carried out over a short time when conditions presumably changed little. In many applications it is impractical to carry out the trapping during a short time because a significant portion of the population must be captured. When data on catch per trap comes from a fishery prosecuted over a season lasting for months, for example, both biological and physical conditions affecting catchability will change. A decrease in average catchability over the season, as from falling temperature, would lead to an underestimate. Increasing catchability, as would happen following a molt, would overestimate the population. These results were supported by simulations (Bratten 1969; Miller and Mohn 1989) which showed large biases in DeLury and Leslie estimates from increased catchability and moderate biases from decreased catchability.

(2) The fishing effort is uniformly distributed over the area of the fished population. This assumption can be reasonably met for a small experimental area where the traps are distributed over the entire area every time they are fished. Small redistribution of the target species or of the traps from one fishing time to the next will expose most of the target animals to traps. This assumption will rarely be met in fisheries where the fishing vessels are much more mobile than the target animals, and the fleet is not large enough to fish all fishable concentrations simultaneously, as is typical of decapod fisheries. There is ample evidence for trap fishermen moving their gear several times during a season in search of higher catch rates (e.g. Mohn and Elner 1987). This behavior can produce a variety of relationships between catch rate and cumulative catch. For example, high density areas might be fished first causing catch per trap to start higher than the average for the population, or areas may be fished in order of increasing distance from port with catch per trap reflecting the densities in these areas. Abundance of Alaskan king crab has been based on preseason trawl surveys and Leslie estimates from the fishery (Otto 1986). Leslie estimates were always lower than trawl estimates and averaged 30% less over 5 yr. The difference was attributed to unequal spatial distribution of fishing effort and low fishing effort on small crabs. The trawl estimates included the assumption that the trawl captured all animals in its path; if this was not true the difference between the two estimates should have been larger.

(3) the quality of the fishing effort, such as soak time, bait type and amount, and trap design, is constant during the time of fishing. These factors can be controlled in experimental fishing, but not in a fishery. If they vary randomly over the season they will not bias population estimates, but a systematic change over time will introduce bias.

(4) The fished population is closed to immigration, emigration, natural deaths, or recruitment. This assumption is most easily met in short-term fishing experiments, and difficult to meet using data collected over a fishing season. Immigration and growth would lead to overestimates, emigration, and natural mortality to underestimates in population size.

What can be used in place of the fishing success methods when there is a good chance that they give a biased answer? No answer to a problem is better than a misleading one. At least then the investigator and his clients understand that the problem has yet to be solved. For fishery derived data, a simple calculation of seasonal mean catch per trap haul requires less daunting assumptions than fishing success methods and performs better in simulations, but gives only a relative measure of population abundance (Miller and Mohn 1989).

The catchability coefficient, the quotient of catch per trap and absolute animal density, is another alternative for density estimates. It does not require a uniform distribution of fishing effort or the capture of a large fraction of the target population, and its assumptions, listed below, are easier to meet than those of the fishing success methods because a time series of catch rates is not required.

(1) Catchability coefficients (q) are applied to population components of homogeneous catchability, e.g. a size or sex.

(2) Application of q's are for the conditions under which they were measured (e.g. temperature or stage of the molt cycle).

(3) the quality of the fishing effort (e.g. bait and trap design) is the same when q's are measured and applied.

(4) The fished population is closed to immigration, emigration, mortality, and recruitment when q is being measured or applied.

Design of Trapping Surveys

The following are considerations when designing a trapping survey. The traps should be affordable and robust, and of a

Table 7. Comparison of catch rates for traps with top and side entrances.

Species	Entrance	Catch/trap	Source
Pandalus platyceros	Long side funnel	5.3	Kessler (1969)
	Medium side funnel with pipe extension	3.8	
	Short side funnel	2.8	
	Top	1.3	
	Side ramp	0.3	
Pandalus borealis	Side funnel	22	Kioke and Ishidoya (1978)
	Top	9	
Homarus americanus	Side funnel	1.1	Stasko (1975)
	Top	0	
Cancer irroratus	Side funnel	5.7	Stasko (1975)
	Top	8.1	

design optimized for a specified size range of a single target species. Attempting to survey a variety of species or a very large size range simultaneously will usually result in not sampling any well. Even within these limits no trap type will catch all components of a population in proportion to their abundance. Therefore, a large effort should be invested in measuring the selectivity biases of the gear. Only after completing this is the question of replication considered. (A tight confidence interval on an incorrect mean is not useful.) Having made a large time investment it will be to our advantage to use the same trap type in the same manner for a long time. If results are to be compared among years or among areas, surveys should be conducted when important factors affecting catchability are similar. For example, American lobster may best be surveyed in the autumn when molting, hatching of old eggs, and extrusion of new eggs are all complete, and the water is warm. Also, high catch rates resulting from high temperatures and postmolt condition would reduce the number of trap hauls needed to achieve a desired confidence interval. Finally, we should be willing to accept that some species and habitats cannot be reliably surveyed with the technology available. These challenges are best left to the next cohort of biologists.

In summary, catch rates by traps remain the most convenient measure of relative, or if q can be measured, absolute population density for most decapod species. Disappointing correlations between catch rate and independent measures of abundance illustrate the difficulty, but the lack of suitable alternatives for routine density measurements justifies continued effort to obtain reliable correlations. Leslie and DeLury methods may be unreliable, especially when applied to data collected over a fishing season. Bias in trapping survey data is a more serious problem than variance.

Trap Design

Entrance

The ideal trap entrance allows the target animal easy access, but prevents escape. Many variations on number, shape, size, and location of entrances have been used to accomplish these ends.

Side entrance traps appear better suited than top entrance traps for catching prawns and American lobster (Table 7). (In the United Kingdom these designs are called creels and pots, respectively). Kessler (1969) fished five trap designs in a tank stocked with 20 prawns. After 4 h, traps with side funnel entrances contained 2.2 to 4.2 times as many prawns as top entrance traps. Among traps with side entrances those with long entrance funnels did best. A trap with a side ramp sloping up to a slit-shaped entrance just under the top of the trap was least successful. Bait location in the bottom of the traps may have discouraged prawns from swimming up to the slit side or the top entrances. Also, escape was high through the slit entrance. In field trials, Koike and Ishidoya (1978) found that traps with side funnels caught 3.5 times as many *Pandalus borealis* as top entry traps. Stasko (1975) compared *Homarus americanus* traps having two side entrances with the same traps with the side entrances covered and a top entrance fitted. The lobster catch dropped to zero in the modified trap while the catch of male *Cancer* crab increased slightly. Thus, the modification effectively excluded lobsters making it possible to use these traps out of lobster season to fish for another target species.

The nearer a funnel entrance was to the bottom of a *Callinectes sapidus* trap the higher the catch (Isaacson 1962). Catch per trap averaged 7.4, 5.3, 0.7, and 0.4 crabs with entrances starting 0, 8, 28, and 38 cm, respectively from the bottom. Presumably, these differences were related to ease of entry.

Diameter of the entrance ring can also affect catch rates by being too small to allow large animals to pass or large enough for easy escape. In an elegant laboratory experiment Matuda et al. (1984) divided a tank into two sections using a fence with a funnel shaped opening in the center. The small end of the opening was 5, 10, 15, or 20 cm wide. Movement of small fish (*Rhodeus ocellatus*) both ways through the entrance, simulating entry and escape, was induced by placing a light over alternate ends of the tank. Entry was over 90% and occurred at the same rate through all four sized openings. However, escapes increased from 4 to 27 to 55 to 78% with each increase in opening size. Using traps fitted with different sized entrance rings, catch rates of *Pandalus platyceros* were approximately (depending on soak time) 2, 10, 4, and 2 prawns per trap with ring diameters of 2.5, 5.1, 7.6, and 10.2 cm, respectively (Boutillier 1985). The author suggested that the smallest ring impeded entry, whereas the largest ones permitted easy escape. When demand dropped for *Homarus americanus* weighing over 4 kg, some traps used in the Canadian offshore fishery were fitted with smaller rings to exclude these jumbo sizes (D. S. Pezzack, Department of Fisheries and Oceans, Halifax, N. S., pers. comm.). In two other studies entrances large enough to allow the target species to pass, restricted entry by unknown means. Catch of *Pandalus borealis* in traps with entrance diameters of 4.3, 8.7, and 11.8 cm averaged 6, 36, and 53 per trap, respectively (Kioke and Ishidoya 1978). Catch of spider crabs (*Chionoecetes japonicus*) with top entrance diameters of 30 and 50 cm averaged 6.4 and 13.9 per trap, respectively (Koike and Ogura 1977).

Triggers, or shutters as they are called in the U. K., have proven effective in reducing escapement from traps with side entrances. Triggers are two vertical wires loosely attached to the top of a rigid trap opening. The wires are pushed up and in when an animal enters and fall down after the animal passes. Since the bottom of the wires extends past the bottom of the entrance they cannot be pushed outward to allow an animal to escape. Sablefish catches in modified king crab traps approximately doubled with the addition of triggers (Hughes et al. 1970). Mason (1965) found approximately a 50% increase in catch of *Cancer pagurus* when traps with 'shuttered eyes' were used. Thomas' (1959) results were more equivocal. Shutters produced no difference in catches of *Cancer pagurus*

or *Homarus gammarus* if entrance tunnels were approximately horizontal, whereas if entrance tunnels sloped upward traps with shutters caught more *H. gammarus* but not more *C. pagurus*. High (1976) placed tagged *Cancer magister* in traps and followed their escape. Only 5% escaped traps with triggers by 12 d compared with 100% escapement from traps without triggers by 3 d.

Simpson (1975) mentioned the effectiveness of traps with 'soft eyes' in preventing escapement of *Homarus gammarus*. The inside opening of the entrance tunnel is not held rigid by a ring, but is made of loose netting that must be pushed open by the lobster on entering. Unfortunately, no comparative data for soft-eyed and hard-eyed traps are given. The same design is used in the tunnel separating the compartments (kitchen and parlor) in American lobster traps (Richards et al 1982; Krouse 1989).

Escape from top entrance traps may be inhibited by a collar of netting for whelks (Hancock 1967), or smooth plastic for decapods. This material hangs down into the trap from the entrance. A whelk or decapod that crawls up the inside of the trap cannot maintain its footing on the barrier and cannot reach the entrance. None of 25 *Cancer productus* or 36 *C. irroratus* escaped traps with collars, whereas 45 of 50 and 21 of 57, respectively escaped traps without (Miller 1978a; 1980).

Probability of entry

T. H. Butler (Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B. C., pers. comm.) stimulated my interest in this area of investigation with field observations that *Cancer magister* always approached traps from down stream and that entry was most likely if the bait odor trail led them to an entrance.

Crab activities in and around baited and unbaited traps were observed in large tanks with the aid of a video camera and a time-lapse recorder (Miller 1978a, b, 1980). Among *Cancer productus* which responded to the bait odor and approached cone-shaped top-entry traps, 27% gained entry. Twenty and 34% of *Cancer irroratus* gained entry to similar traps in two other studies. The success rate of a spider crab, *Hyas araneus*, entering cone-shaped traps was lowest at 12%. When a side-entry trap was set with entrances parallel to the current such that the bait odor trail led *C. productus* directly to the entrance, 65% of approaches resulted in entry. However, when the same trap was turned 90°, and the entrance was about 30 cm from the odor trail, the success rate dropped to 7%.

Another data set from this series (Fig. 6) also shows the dramatic effect of orientation of side-entry traps. With a two-entrance trap baited in the center, entry success of *C. irroratus* ranged from 70 to <10%. Averaged over all orientations the capture success of two entrance-traps, whether they had one or two baits, were 31–33%. Succcess in entering a three-entrance trap ranged from 27 to 70% and averaged 49% over all orientations. The third entrance increased the probability of entry because an entrance was more likely to be facing downstream. If the odor trail did not lead crabs to the entrance they attempted to enter through the mesh at the point where the odor was strongest. As crabs became attracted to this location they spent considerable time in aggressive encounters. When they found an entrance, it appeared to be more by chance than from a systematic search. Crabs showed little interest in unbaited traps.

Bjordal (1986) used underwater video to observe the behavior of Norway lobster (*Nephrops norvegicus*) around a side-entry trap in the field. Only 9% of lobsters who touched the trap entered. Orientation of the entrances to the current was not noted, but difficulty in locating an entrance and frequent aggressive encounters were noted.

Karnofsky and Price (1989) accumulated 103 h of observations of trap-related behavior of American lobster in a large tank. Only 11% of lobsters which touched the trap entered and 76% of these escaped through the entrance, leaving only 2.6% captured after a 24-h soak. Traps were conventional wood-lath parlor traps oriented so the center of the bait odor trail left the trap 10–20 cm from the entrance. As in the above studies, agonistic encounters were frequent. Jernakoff and Phillips (1988) found a much higher capture rate for electromagnetically-tagged *Panulirus cygnus* in the field. Sixty percent of those which reached the trap overnight were found in the trap the next morning. Because the traps had horizontal top entrances, their orientation to the current was not relevant.

The bait odor from a trap of ideal design would always lead the target animal to the entrance. This would occur if the trap was always oriented with the entrance downstream of the bait, or if the bait odor could only leave the trap through the entrance. I am not aware of a method of achieving this under most fishing conditions. However, it could be achieved if current direction did not change over the time of fishing and the fishermen were able to orient the trap correctly. Another solution, used by Sutterlin (1975), is useful under conditions of low wave influence, strong current, and a smooth bottom. A rope tether connects a bucket-shaped trap to an anchor, enabling the current to roll the trap until the funnel entrance faces downstream.

Size Selectivity by Escape Gaps and Mesh

Escape gaps retain commercial sized animals while allowing subcommercial sizes to escape. Advantages of escape gaps in American lobster traps were summarized by Templeman (1958) as: (1) fewer sublegals are caught by fishermen who sell them illegally; (2) fewer sublegals will be injured by handling, exposure to air and sunlight, and exposure to larger lobsters in traps; (3) fewer sublegals will die in lost traps; (4) fewer sublegals will be exposed to predators by returning them to the water away from a shelter in daytime; (5) traps can be fished more quickly because fewer sublegals and nuisance species, such as crabs, need to be removed; (6) traps might be less expensive to build because larger openings require less material.

Selectivity experiments conducted by placing lobsters or crabs of known size in traps with escape gaps have shown that the size selection can be precise. For example, no American lobsters 81 mm CL, but 80% of lobsters 79 mm CL passed through a round opening of 58 mm (Krouse 1978). Different sized escape gaps might be optimum for different populations of lobsters, or for males and females, because the lobster carapace length (the dimension determining legal size): carapace width (the dimension determining selectivity) ratios can differ (Fogarty and Borden 1980; Maynard et al. 1987; D. Maynard, Department of Fisheries and Oceans, Moncton, N.B., pers. comm.) The rigid escape gap openings, the rigid exoskeleton of decapods, and the dexterity of decapods to orient themselves to the most advantageous position for escape no doubt account for the precision in selectivity. Stasko (1975) provided the useful generalization that *Cancer irroratus* and *Homarus americanus* oriented themselves such that the smallest opening an animal could be pushed through by hand was also the smallest opening it could pass through unaided.

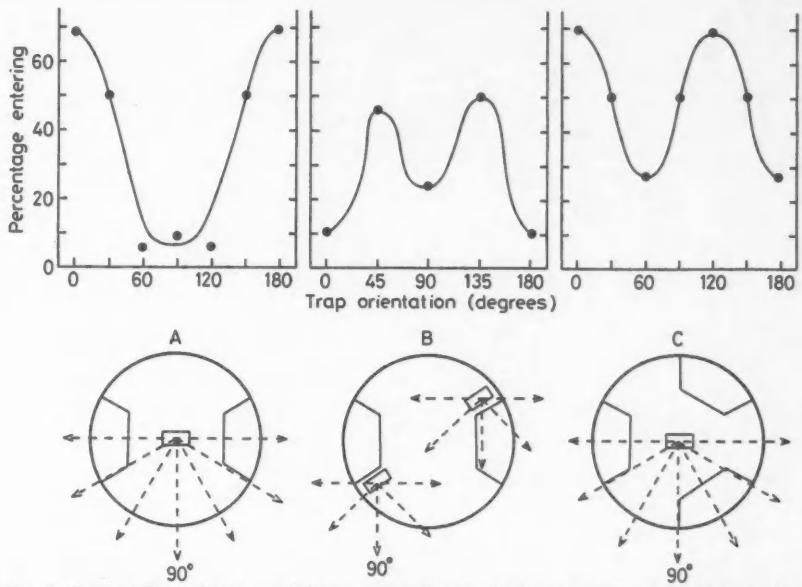

FIG. 6. (Bottom) Trap orientation relative to current direction for A, two-entrance trap with center bait; B, two-entrance trap with baits near entrances; and C, three entrance trap with center bait. Top. Percentage of crabs that approach which gain entry when current directions are as shown at the bottom of the figure (from Miller 1980, J. Cons. Int. Explor. Mer 39: 140–147, figure 3).

The success of escape gaps for a variety of species, trap types, catch rates, and environmental variables may be surprising considering that the captured animal must locate the escape gap and voluntarily exit. The ratios of the number of subcommercial to commercial sized crabs or lobsters captured in traps with different sized escape openings are given in Table 8 for a large sample of field studies. For *Homarus* sp. the ratio of subcommercial to commercial catch was 2 to 21 times greater in traps with small than large escape openings, i.e. the capture of undersized animals was 2 to 21 times greater. For crabs (*Cancer, Chionoecetes, Callinectes*) this ratio varied from 1.7 to 12. Not included in Table 8 are extensive results of mesh selectivity for *Pandalus borealis* and *Chionoecetes japonicus* reported in Kioke and Ogura (1977). Size selectivity of woven mesh openings would be affected by the tightness of the mesh on the trap frame and by the shape of the opening. Because mesh openings are flexible, one would expect selectivity not to be as precise as for rigid openings.

The popular view that catch per trap of commercial sizes increases if more subcommercial animals are permitted to escape (Krouse and Thomas 1975; Fogarty and Borden 1980; Brown 1982; Weber and Briggs 1983; many Canadian fishermen and fisheries managers, pers. comm.), was not confirmed by several of the studies reviewed in Table 8 (see column with number of legals per trap haul). In some studies escape gaps may have been large enough for commercial sizes to escape, or the experimental designs may simply not have been sensitive to the accuracy and precision of commercial catch per trap. Nevertheless, enhancement of the commercial catch should be demonstrated for a local fishery and gear type before this benefit is claimed for escape gaps.

Ghost fishing

Traps ghost fish when they capture and retain the target species after being lost or abandoned by fishermen. Ghost fishing can be divided into a short phase of high catch rates when the bait is an attractant, and a longer phase of low catch rates after the bait looses its attraction. A trap stops ghost fishing when it is recovered, broken up by storms, or degrades to a state that all captured animals can escape. The number of animals lost to ghost fishing depends on the number of traps lost, how long the traps continue to fish, and the rate of entry and escape. Mortality caused by ghost fishing may always be a small fraction of landings, but solutions can be inexpensive, and thus cost effective.

Ghost fishing mortality in Dungeness (*Cancer magister*) traps can be important even though some crabs are able to escape around the trigger wires in the entrance and through escape openings in the sides. Breen (1987) set 10 baited Dungeness traps in an area of low commercial abundance, and observed them by diving 18 times over the next 12 mo. From the cumulative mean catch of 16.9 crabs/trap, 21% were caught in the first 2 wk and 79% during the remainder of the year. Fifty-five percent of the catch died, 27% escaped, and 18% remained at the end of the year. The trap lids were then unlatched and 76% of the remaining crabs escaped, showing that a timed release device would be effective. Breen applied these ghost fishing mortalities to losses of traps in another area and estimated that crab losses to ghost fishing were about 7% of annual landings. High (1976) stocked traps with Dungeness crabs and observed them by diving 12 times over 74 d. Twenty-one percent escaped and 26% died. The triggers were then raised and 92% of those remaining escaped within 3 d. Muir et al. (1984) baited traps daily for 28 d but did not remove the catch. Even over this short time 19% of the Dungeness died. Demory (1971) recovered 117 Dungeness traps that had not been fished for 6 wk or longer. They contained 3.2 t of this valuable species.

Pecci et al. (1978) set baited two-compartment American lobster traps on three occasions and observed them by diving

TABLE 8. Ratio of sublegal to legal sized decapods, and the number of legal sized decapods, caught in traps with different sized escape openings. Openings are rigid unless labeled as mesh.

Species	Opening size (mm)	Minimum legal size (mm)	Number caught	Number sublegal: number legal	Number legal (trap haul)$^{-1}$	Source
Homarus americanus	45 × >300	79 CL	497	0.3:1	1.6	Templeman (1939)
	34 × >300		1034	1.7:1	1.6	
	44 × >300	79 CL	1675	0.4:1	[a]	Wilder (1949)
	32 × >300		3015	1.5:1	[a]	
	32 × >300	61 CL	10 240	0.3:1	[a]	
	25 × >300		13 595	0.8:1	[a]	
	44 × 152	81 CL	289	0.8:1	0.35	Krouse and
	41 × 152		333	1.7:1	0.23	Thomas (1975)
	38 × 152		281	5.1:1	0.15	
	25 × 25		2004	16.4:1	[a]	
	44 × 152	81 CL	305	0.6:1	0.55	Krouse (1978)
	~32X × 300		749	4.3:1	0.53	
	58 circle	81 CL	584	2.2:1	0.64	
	~32 × >300		852	4.8:1	0.53	
	44 × 152	81 CL	499	3.0:1	0.35	Pecci et al. (1978)
	~30 × >300		1349	7.8:1	0.42	
	44 × 152	81 CL	454	2.8:1	[a]	Nulk (1978)
	25 × 25		1304	7.7:1	[a]	
	42 × 152	78 CL	7840	1.4:1	0.95	Fogarty and
	~31 × >300		11 117	2.8:1	0.85	Borden (1980)
	44 × 152	78 CL	792	0.4:1	0.87	
	42 × 152		1270	1.4:1	0.88	
	~31 × >300		1196	3.1:1	0.94	
	44 × >300	76 CL	246	0.3:1	0.84	Monette (1982)
	~30 × >300		523	0.9:1	0.95	
	58 circle	81 CL	512	0.2:1	0.38	Weber and
	~37 × ~37		472	0.4:1	0.30	Briggs (1983)
	44 circle	63 CL	512	0.2:1	[a]	Maynard et al.
	37 × 450		717	0.7:1	[a]	(1987)
	32 × 450		432	1.2:1	[a]	
Homarus gammarus	42 × 74	80 CL	46	0.5:1	0.18	Brown (1982)
	~32 × ~32		117	4.3:1	0.13	
Cancer magister	108 circle	160 CW	114	2.1:1	1.1	Jow (1961)
	~50 × ~50		233	5.3:1	1.1	
	114 circle	160 CW	237	0.1:1	1.9	
	108 circle		418	0.9:1	1.9	
	102 circle		577	1.3:1	2.3	
	109 circle	155 CW	224	2.6:1	0.98	Smith and
	103 circle		345	3.6:1	1.19	Jamieson (1909)
	~65 × 65 mesh		582	11.9:1	0.71	
Cancer irroratus	58 circle	90 CW	93	1.5:1	4.2	Krouse (1978)
	~32 × >300		265	2.5:1	1.4	
Cancer pagurus	38 × 74	115 CW	759	1.2:1	2.5	Brown (1982)
	32 × 32		1711	4.5:1	2.2	
Chionoecetes opilio	66 × 55 mesh	102 CW	2256	1.2:1	9.2	Miller (1976)
	55 × 55 mesh		2895	1.5:1	9.7	
	46 × 46 mesh		3422	1.8:1	10.4	
	75 × 75 mesh	90 CW	101	0.3:1	4.4	Sinoda et al.
	50 × 50 mesh		123	0.6:1	4.3	(1987)
	15 × 15 mesh		173	1.1:1	4.6	

TABLE 8. *(continued)*

Species	Opening size (mm)	Minimum legal size (mm)	Number caught	Number sublegal: number legal	Number legal (trap haul)$^{-1}$	Source
Callinectes sapidus	38 × 57	127 CW	1140	0.1:1	[a]	Eldridge et al. (1979)
	38 × 54		1155	0.2:1	[a]	
	~38 × ~54 mesh		1459	0.4:1	[a]	
	64 circle	127 CW	761	0.1:1	13.8	
	~38 × ~45 mesh		1059	0.6:1	13.2	
Pandalus platyceros	22 × 22 mesh	30 CL	105	0.2:1	2.4	Boutillier and Sloan (1988)
	12 × 12 mesh		175	1.3:1	1.9	
	24 × 24	30 CL	75	0:1	1.8	
	12 × 24		167	0.8:1	2.4	

[a]Data not available.

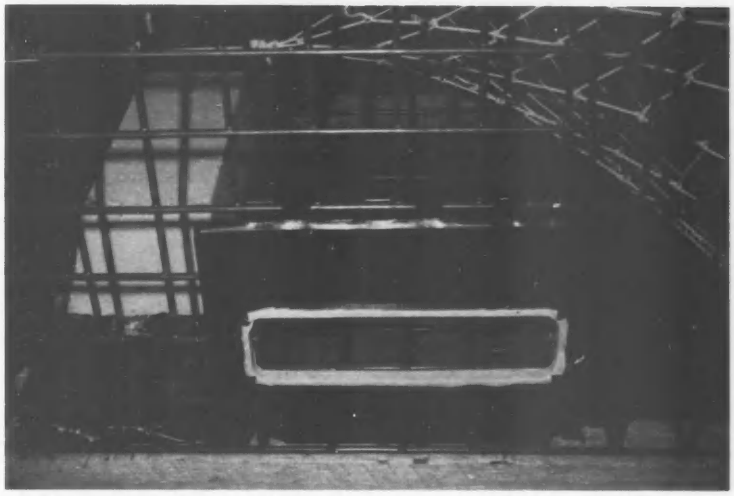

FIG. 7. Wire American lobster trap with combination escape gap and timed release panel to prevent ghost fishing (first described by Blott 1978).

TABLE 9. Catch rates, number per square metre of trap bottom area, for different trap sizes.

Species	Trap characteristics	Trap bottom area (m^2)	Catch per m^2	Source
Tropical reef fish (kg)	Side entrance, escape possible	1.3	2.6	Munro (1974)
		2.1	2.5	
		2.8	2.9	
Cancer productus	Top entrance, escape possible	0.24	13	Miller (1978a)
		0.48	30	
		0.64	45	
Chionoecetes opilio	Top entrance, escape possible	1.2	36	Lafleur et al. (1983)
		2.2	27	
Pandalus platyceros	Side entrance, escape possible	0.25	35	Boutillier and Sloan (1987)
		0.45	40	
		0.74	29	

for 78 to 97 d each. During February–May, 32 lobsters entered 15 traps during the first week, and 16 entered subsequently. Seven of these died, seven were missing (died or escaped), and 34 remained at the end of the observation. During the warmer months of May–August, 148 lobsters entered 31 traps during the first week and 171 entered subsequently. Of these, 58 died, 119 were missing, and 102 remained. Based on these data Smolowitz (1978) calculated annual mortality from ghost fishing at 1.1 kg/trap without an escape opening, and 0.7 kg/trap with an escape opening. Total loss would be on the order of 3 to 6% of annual landings for the United States fishery.

King crabs (*Paralithodes camtschatica*) successfully escaped

(80–92% in 16 d) from side entry traps, and infrequently entered unbaited traps (High and Worlund 1979), suggesting that a timed release opening is unnecessary for this gear.

Sablefish (*Anoplopoma fimbria*) traps fished in western Canada are required by regulation to have a section of the sidewall secured with untreated natural fiber which on deterioration produces an opening not less than 20 × 20 cm. Traps with an opening of this size had only 10% of the catch of control traps (Scarsbrook et al. 1988).

Losses to ghost fishing can be reduced by fishing fewer traps, fitting traps with openings to allow sublegal animals easy escape, fitting traps with biodegradable sections (untreated wood or natural fiber netting), or fitting traps with panels attached with corrodible (Smolowitz 1978) or biodegradable (Scarsbrook et al. 1988) fasteners. Fitting a combination escape gap-ghost fishing panel (Fig. 7) adds about U. S. $0.50 to the cost of an American lobster trap (pers. observ). Attaching the panel to the trap with corrodible fasteners that need to be changed annually would require a small amount of labor, but negligible material cost.

Trap Size

Four studies have shown that larger traps yield larger catches; however, the amount of increase was not consistent. The catch of tropical reef fish was proportional to trap size, the catch of *Cancer* crabs increased more than trap size, and catches of spider crabs and prawns increased less than trap size (Table 9).

Munro (1974) hypothesized that fish escaped more easily from small traps, and Miller (1978a) demonstrated this experimentally for *Cancer* crabs. However, in traps from which escape was not possible catches of spider crabs (*Chionoecetes opilio*) were still greater in large than small traps (Lafleur et al. 1983). Reduced saturation effect in larger traps may be the mechanism responsible. That is, if the captured animals produce an odor or sound that discourages further entry, dispersal of the catch over a larger trap would reduce the stimulus concentration.

Traditional designs

Decapod catches by traps of two or more traditional designs have often been compared in field trials, either as a service to fisherman or to standardize catch per unit effort to a single trap type. However, since traps being compared differed in more than one design feature, the feature responsible for differences in catch rates could not be positively identified.

Two-chambered American parlor traps were superior to one-chambered creels in all comparisons for the three species tested (Table 10). The second chamber (parlor or bedroom) is entered through a funnel-shaped opening from the first chamber (kitchen). The second chamber greatly reduces the chance of escape and holds the captured animals away from the bait, possibly reducing the saturation effect as well. The benefit of the parlor was also demonstrated during observations on parlor traps only. R. A. Richards (U. S. National Marine Fisheries Service, Woods Hole, Mass., pers. comm.) placed American lobsters in the parlor of traps and observed the number remaining after 24 h. Fifty-four traps in tanks were stocked with eight lobsters each, only 3 (<1%) escaped. In the field three lobsters were stocked in each of 63 traps and eight lobsters in each of 50 traps. In both cases only 5% escaped. Karnofsky and Price (1989) noted that 26 of 34 American lobsters which entered the kitchen (an unspecified number of these kept their telson over

TABLE 10. Comparison of decapod catch rates among traps of traditional designs.

Trap type	Entrance	Number of chambers	Relative catch	Source
Am. parlor	Side	2	1.00[a]	Gibson and Heffernan (1967)
Scottish creel	Side	1	0.44[a]	
French creel	Side	1	0.20[a]	
Igloo	Top	1	0.10[a]	
Am. parlor	Side	2	1.00[a] 1.00[b]	Lovewell et al. (1988)
Scottish creel	Side	1	0.46[a] 0.72[b]	
Am. parlour	Side	2	1.00[c]	DeBruin (1960)
Scottish creel	Side	1	0.68[c]	
Inkwell	Top	1	0.47[c]	
Scottish creel	Side	1	1.00[b]	Shelton and Hall (1981)
Inkwell	Top	1	0.91[b]	
Scottish creel	Side	1	1.00[a] 1.00[b]	Thomas (1954)
Cornish pot	Top	1	0.73[a] 0.94[b]	
Rectangular	Side	1	1.00[d]	Lafleur et al. (1983)
Conical	Top	1	0.73[d]	
Rectangular	Top	1	1.00[e]	Wenner et al. (1987)
Boat shaped	Side	1	0.66[e]	
Am. parlor, wood	Side	2	1.00[f]	Keser et al. (1983)
Am. parlor, wire	Side	2	0.96[f]	

[a]*Homarus gammarus*
[b]*Cancer pagurus*
[c]*Panulirus* sp.
[d]*Chionoecetes opilio*
[e]*Geryon fenneri*
[f]*Homarus americanus*

TABLE 11. Means of capturing and retaining desirable species and sizes, and not capturing or releasing undesirable species and sizes.

Point of selection	Selection for desirable catch	Selection against undesirable catch
Odor detection	Bait attracts and is long lasting	Bait repels
Enter	Bait odor exits through entrance	
	Entrance easy to enter Saturation effect reduced	Entrance difficult to find, or its size or shape prevents entry
Escape through entrance	Entrance hard to find Entrance closed to exit	
Escape elsewhere	Mesh or escape port too small	Mesh or escape port large enough, Escape port easy to find and exit

the entrance ring) escaped, whereas none of eight which entered the parlor during 24 h escaped.

As summarized in Table 10, catches by creels and top entry traps (igloo, inkwell, Cornish) were similar in four of five comparisons. Rectangular side-entry traps caught marginally more snow crabs than did conical top-entry traps of the same bottom area. A rectangular top-entry trap caught marginally more *Geryon* than a boat-shaped side-entry trap of similar size. Catches of American lobster were the same in wire and wood parlor traps.

The blue crab (*Callinectes sapidus*) trap is divided into upper and lower chambers with a horizontal partition. Crabs enter and feed at the bait in the lower chamber, then pass through a conical opening to the top chamber (Van Engle 1962). The advantage of the second chamber are presumably the same as for the parlor in the American lobster trap. Only 5% of crabs placed in the top chamber escaped, whereas 48% escaped from traps without a partition (Isaacson 1962).

The Ideal Trap

Regier (1971, University of Toronto, Toronto, Ont., pers. comm.) proposed a model for partitioning the probability of capture, $P(C)$, into four elements

$$P(C) = P(E)\,P(I)\,[1 - P_1(X) - P_2(X)]$$

where $P(E)$ is the probability of encountering a trap, $P(I)$ is the probability of entering given encounter, $P_1(X)$ is the probability of escape through the entrance, and $P_2(X)$ is the probability of escape through another part of the trap (e.g. escape opening). In the ideal trap the probability of entry would be high and the probability of escape low for desired sizes of the target species, whereas the opposite would occur for subcommercial sized individuals or noncommercial species.

Table 11 provides a summary of objectives for catch selectivity by bait and trap design. A good bait will produce an odor that is attractive at low concentration, that remains attractive for the duration of the soak, and that is not entirely consumed by the catch. A fisherman may also want the bait to repel undesirable species, such as a dead crab to repel conspecifics. In the ideal trap the bait odor trail leads the target animals to the entrance and the entrance is easy to enter. On the other hand, an entrance location which is difficult to find or a size which is difficult to enter can be useful for excluding undesirable animals. Catch limitation due to saturation effects (animals inside decreasing entry) can be reduced by increasing trap size, soak time, and bait quantity, and probably by keeping captured animals away from the entrance and bait.

Escape of desirable catch through the entrance has been reduced by placing the entrance away from the catch (e.g. above the bottom), adding a second compartment that holds the catch away from the entrance, making the opening as small as practical, adding trigger wires that only open inward, or adding material around the entrance that is difficult to crawl over. Size selectivity can be regulated by the mesh size, and size and shape of the escape port.

Two major improvements remain for the design of the ideal decapod trap. If bait odor could be made to exit only through the trap entrance, traps would fish much faster, that is, reach the asymptotic catch level sooner, and permit fishermen to use shorter soak times and fewer traps. Second, it has been shown that the asymptotic catch level may not be limited by availability of the target species, or by the trap's capacity, but by a repelling stimulus that the captured animals release. If this stimulus could be masked or prevented, catch per trap haul might increase significantly.

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ANNOUNCEMENTS/AVIS

Conference on Aquatic Systems in Semi-Arid Regions : Implications for Resource Management

Saskatoon, Saskatchewan
27–30 August 1990

The National Hydrology Research Institute and the Rawson Academy of Aquatic Science are pleased to host this conference which is dedicated to the memory of Prof. D. S. Rawson (1905–1961), an internationally recognized scholar and pioneer in aquatic science. The objective is to bring together aquatic scientists, resource experts, and managers with an interest in aquatic environments in semi-arid regions, to examine existing knowledge of these systems and the options and opportunities for their effective management. Semi-arid regions encompass a variety of aquatic systems ranging from oligotrophic to hypereutrophic freshwater lakes; and, from saline lakes and inorganically turbid reservoirs to fragile wetlands.

Preliminary Program:

26 August	Registration
27 August	Rawson award and lecture

27–28 August:
Aquatic Environments — saline and freshwater lakes, in organically turbid systems, rivers, wetlands and sloughs :
- chemical processes (nutrient dynamics and contaminants)
- physical processes (sediment erosion)
- biological processes (trophic interactions and community structure)

28–29 August:
Management of Aquatic Environments:
- agricultural, industrial and urban impacts
- climate change and drought
- models
- case studies

Aquatic Sciences and Water Management:
- panel and participant discussion

30 August:
Field trips to local saline and boreal forest lakes.

Further information available from: Scientific Information Division, National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, Saskatchewan, Canada S7N 3H5 Telephone: (306) 975-4022 FAX: (306) 975-5143.

Conférence sur les systèmes aquatiques des régions semi-arides: Répercussions sur la gestion des ressources

27 au 30 août 1990
Saskatoon (Saskatchewan)

L'Institut national de recherche en hydrologie et l'Académie Rawson des sciences de l'eau ont le plaisir de parrainer cette conférence dédiée à la mémoire du professeur D. S. Rawson (1905–1961), un intellectuel de réputation internationale qui a été pionnier des sciences de l'eau. Son objectif consiste à réunir les scientifiques du domaine hydrique, ainsi que les experts et gestionnaires en ressources s'intéressant aux milieux aquatiques des régions « semi-arides » pour examiner l'état des connaissances des ces systèmes, ainsi que les options et perspectives de gestion efficace. Les régions dites « semi-arides » comprennent une foule de système aquatiques allant des lacs d'eau douce oligotrophiques à hypereutrophiques, aux lacs salins, aux réservoirs à turbidité inorganique et aux terres humides fragiles.

Programme Préliminaire

Le 26 août	Inscription et rencontre
Le 27 août	Prix Rawson et conférence

Le 27 et 28 août:
Les milieux aquatiques - les lacs salins et d'eau douce, les systèmes à turbidité inorganique, les rivières, les terres humides et les bourbiers :
- les processus chimiques (dynamique des éléments nutritifs et des contaminants)
- processus physiques (érosion sédimentaire)
- processus biologiques (interactions trophiques et structure communautaire

Les 28 et 29 août:
Gestion des milieux aquatiques:
- Répercussions agricoles, industrielles et urbaines
- Variations climatiques et sécheresse
- Modélisation
- Études de cas

Les sciences aquatiques et la gestion de l'eau
- Discussion entre experts et avec les participants.

30 août:
Expéditions jusqu'à des lacs salins et de la forêt boréale.

De plus amples informations seront disponibles de l'adresse suivant : Division d'information scientifique, Institut national de recherche en hydrologie, 11, boulevard Innovation, Saskatoon (Saskatchewan) Canada S7N 3H5 N° de téléphone: (306) 975-4022 N° de télécopieur: (306) 975-5143.

Aquatic Toxicity Workshop

The 17th Annual Aquatic Toxicity Workshop will be held at the Hotel Vancouver, Vancouver, British Columbia, on November 5–7, 1990. The theme of the Workshop will be Threshold Biological Responses: Predicting and Determining Ecological Relevance. Major topics will include (i) methodology and development: the tools and the practice, (ii) histopathological responses: so what if fish have lesions, (iii) genotoxic/cytotoxic responses, (iv) acute versus chronic responses, (v) exotic chemicals: managers, scientists and the public, (vi) toxicology and sustainable development, and (vii) toxicological and industrial regulation: new approaches and procedures. A plenary session will be held on Predicting and Monitoring Biological Effects. Focus of the Workshop will be on major water bodies of concern including the Fraser River, the St. Lawrence, Halifax Harbor, Vancouver Harbor, and Puget Sound. Platform and poster presentations will be accepted in English or French, and submissions must be received by August 31, 1990 to be considered. For further information, please contact Dr. Peter M. Chapman, Program Chair, c/o E.V.S. Consultants, 195 Pemberton Avenue, North Vancouver, British Columbia, V7P 2R4 Canada. Telephone (604) 986–4331. FAX (604) 662-8548.

Atelier annuel sur la toxicité aquatique

Le 17e Atelier annuel sur la toxicité aquatique se tiendra du 5 au 7 novembre 1990 à l'hotel Vancouver, à Vancouver (Colombie-Britannique). L'atelier aura pour thème: les réponses biologiques seuils: prévision et détermination de la pertinence écologique. Les principaux sujets traités seront: i) La méthodologie et le développement: les outils et la pratique; ii) Les réponses histopathologiques: les lésions des poissons; iii) Les réponses à la génotoxicité et à la cytotoxicité; iv) Les réponses aiguës ou chroniques; v) Les produits chimiques spéciaux: les gestionnaires, les scientifiques et le public; vi) La toxicologie et le développement soutenu et vii) Toxicologie, industrie et réglementation: approches et procédures nouvelles. Il y aura une séance plénière qui portera sur la prévision et le contrôle des effets biologiques. Les travaux de l'atelier seront surtout axés sur les principaux plans d'eau sources d'inquiétudes, notamment les fleuves Fraser et Saint-Laurent, les ports d'Halifax et de Vancouver et le détroit Puget. Les présentations, sous forme d'exposés ou d'affiches, peuvent être soumises en Anglais ou en Français et doivent être reçues au plus tard le 31 août 1990. Des renseignements supplémentaires peuvent être obtenus auprès du Dr. peter M. Chapman, responsable du programme, a/s E.V.S. Consultants, 195 avenue Pemberton, North Vancouver, Colombie-Britannique, V7P 2R4. Tél.: (604) 986–4331, FAX: (604) 662–8548.

Canadian Conference for Fisheries Research (CCFFR)

University of Guelph
Guelph, Ontario
January 3–4, 1991

Announcement of Conference and Call for Papers

This conference will be held in Guelph, Ontario, January 3 and 4, 1991.

The major themes of the conference will be :

1. Biodiversity at or Below the Species Level

(Convenors : Moira Ferguson, John Leatherland, and Glen van der Kraak)

Topics:

a) Biodiversity at the species level: disappearing taxa or the fate of non-exploited fishes;

b) Quantifying biodiversity below the species level: stock identification, stock definition, etc.;

c) Maintaining biodiversity: technology used for gene bank development, value of gene banks, hatchery breeding programmes;

d) Increasing biodiversity: stock choice, habitat restoration;

e) Decreasing biodiversity: effect of stock transplantation, effect on endemics, etc.;

f) Manipulating biodiversity: use of triploids, sex manipulation, transgenic studies, etc.

2. Sustainable Fisheries

(Convenors : W. Jack Christie, David L. G. Noakes)

Topics:

a) Sustainable by and to whom? Assessing costs and benefits;

b) Environmentally sustainable fisheries;

c) Politically sustainable fisheries; and

d) Economically sustainable fisheries.

While papers on other themes may be submitted, authors are encouraged to adhere to the above themes as much as possible.

Deadline for abstracts is August 30, 1990. They should be typed (maximum 200 words, single spaced) and nature of presentation (oral/poster) indicated. A high quality of copy is required, suitable for photographic reproduction.

Conférence Canadienne sur la recherche sur les pêches (CCRP)

Université de Guelph
Guelph, Ontario
les 3 et 4 janvier 1991

Avis de conférence et demande d'articles

Cette conférence aura lieu les 3 et 4 janvier 1991 à Guelph, Ontario.

La conférence portera sur les principaux thèmes suivants:

1. La biodiversité au niveau de l'espèce ou de la sous-espèce

(Responsables : Moira Ferguson, John Leatherland et Glen van der Kraak)

Sujets:

a) La biodiversité au niveau spécifique: les taxons en disparition ou le destin des espèces non exploitées;

b) La quantification de la biodiversité au niveau infra-spécifique : l'identification des stocks, la définition d'un stock, etc.;

c) La conservation de la biodiversité: les techniques utilisées pour la création de la banque de gènes, la valeur de ces banques, les programmes de reproduction en écloserie;

d) L'augmentation de la biodiversité: le choix de stock, la restauration des habitats;

e) La réduction de la biodiversité: l'incidence de la transplantation de stock, l'incidence sur les espèces endémiques, etc.;

f) La manipulation de la biodiversité: l'utilisation d'individus triploïdes, la manipulation génétique, les études transgéniques, etc.

2. Rendement des pêches à l'équilibre

(Responsables : W. Jack Christie, David L. G. Noakes)

Sujets:

a) Qui en sera responsable et qui en bénéficiera? Une évaluation des coûts et des avantages;

b) Des pêches viables au niveau environnemental;

c) Des pêches viables au niveau politique;

d) Des pêches viables au niveau économique.

On peut présenter des études sur d'autres thèmes quoiqu'on encourage les chercheurs à s'en tenir autant que possible aux thèmes susmentionnés.

La date limite d'envoi de résumés a été fixée au 30 août 1990. La résumé doit être dactylographié (200 mots au maximum, simple interligne) et l'on doit préciser le genre de présentation) présentation orale/affiche). Une copie de très bonne qualité est requise pour faciliter la reproduction photographique.

Abstracts should be addressed to :
Dr. Eugene K. Balon
Department of Zoology
University of Guelph
Guelph, Ontario N1G 2W1
Tel. (519) 824-4120, ext. 3978

Veuillez faire parvenir votre résumé à:
Dr Eugene K. Balon
Département de Zoologie
Université de Guelph
Guelph (Ontario) N1G 2W1
Tel. (519) 824-4120, poste 3978

International Conference on the Biological Interactions of Enhanced and Wild Salmonids

June 17 – 20, 1991.
Nanaimo, British Columbia, Canada

Programs to increase fish production through artificial manipulations should be evaluated for the change in total production of both enhanced and wild stocks. The production from enhancement programs can generally be evaluated. However, their impact on production from wild stocks is seldom known, although the existence of interaction between enhanced and wild stocks is now broadly postulated. The purpose of this conference is to encourage discussion on potential interactions between enhanced and wild salmonids and on an objective perspective of increasing fish production through enhancement. We propose to discuss:

— production trends and biological characteristics of enhanced stocks with long production histories;
— genetic changes as a consequence of enhancement;
— limits to potential production in both freshwater and marine environments due to predation, competition, or disease;
— fishery management of mixed enhanced and wild stocks and/or species.

Invited speakers will probably address the first and last topics, encompassing a broad geographic range. The remaining two will be dealt with in contributed papers and posters. It is proposed to publish proceedings in the Can. Special Publ. Aquat. Sci. series, after peer review.

If you wish to participate or to receive further information, please contact : *Dr. B. Riddell, Chairman Organizing Committee, BIEWS, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6*. FAX: (604) 756-7053 or phone Ms. Ann Thompson (604) 756-7260.

Conférence internationale sur l'interaction biologique des salmonides améliorés et salmonidés sauvages

17 au 20 juin 1991
Nanaïmo, en Colombie-Britannique (Canada)

Il y aurait lieu d'évaluer les programmes conçus pour accroître la production de poisson par divers moyens d'intervention afin d'en déterminer l'effet sur la production globale des stocks de poissons améliorés et de poissons sauvages. S'il est vrai que la production résultant des programmes de mise en valeur peut habituellement être évaluée, son incidence sur la production de poissons issus de stocks naturels est rarement connue, bien que l'hypothèse d'une interaction entre les stocks améliorés et naturels soit largement admise aujourd'hui. La conférence aura pour objet de stimuler un débat sur l'interaction qu'il peut y avoir entre les salmonidés améliorés et les salmonidés sauvages et d'examiner de façon objective la possibilité d'accroître la production de poisson grâce à des techniques de mise en valeur. Nous proposons de porter les sujets suivants à l'ordre du jour :

— Tendances de la production et caractéristiques biologiques de stocks améliorés qui ont été suivis pendant plusieurs générations;
— Transformations génétiques engendrées par les mesures de mise en valeur;
— Limites que la prédation, la concurrence et la maladie imposent quant aux possibilités de production en eau douce et en eau salée;
— Gestion des pêcheries peuplées de stocks de poissons sauvages et améliorés ou de différentes espèces.

Les conférenciers invités traiteront probablement des premiers et dernier sujets, et ce, dans l'optique de différentes régions géographiques. Les deux autres questions seront abordées dans les exposés soumis et les affiches. Les organisateurs se proposent de faire publier les délibérations de la conférence dans la série des publications spéciales canadiennes des sciences halieutiques et aquatiques, après évaluation par d'autres spécialistes du domaine.

Si vous désirez participer à la conférence ou obtenir de plus amples informations, prière de communiquer avec *M. B. Riddell, président du Comité d'organisation, IBSASS, Station biologique du Pacifique, Nanaïmo (C.-B.), Canada V9R 5K6*. [Télécopieur : (604) 756-7053], ou d'appeler M^me^ Ann Thompson au (604) 756-7260.

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